Simulation, Models, and Refactoring of Bacteriophage T7 Gene Expression

by

Sriram Kosuri

B.S. Bioengineering
University of California, Berkeley, 2001

SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL ENGINEERING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTER OF SCIENCE IN BIOLOGICAL ENGINEERING
AT THE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

JANUARY 2007
[February 2007]

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Signature of Author: _____
Division of Biological Engineering
January 10, 2007

Certified by: _____
Drew Endy, Ph.D.
Assistant Professor of Biological Engineering
Thesis Supervisor

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This thesis has been accepted by a committee of the Biological Engineering Division as follows:

Professor Bruce Tidor

Professor of Biological Engineering
Professor of Electrical Engineering & Computer Science
Thesis Committee Chairman

Professor Drew Endy

Cabot Assistant Professor of Biological Engineering
Thesis Adivisor

Professor Douglas Lauffenburger

Whitaker Professor of Biological Engineering
Professor of Chemical Engineering
Professor of Biology

Dr. Tom Knight

Senior Research Scientist
Computer Science and Artificial Intelligence Laboratory

Professor Ian Molineux

Professor of Molecular Genetics and Microbiology
University of Texas, Austin

Abstract

Our understanding of why biological systems are designed in a particular way would benefit from biophysically-realistic models that can make accurate predictions on the time-evolution of molecular events given arbitrary arrangements of genetic components. This thesis is focused on constructing such models for gene expression during bacteriophage T7 infection. T7 gene expression is a particularly well suited model system because knowledge of how the phage functions is thought to be relatively complete. My work focuses on two questions in particular. First, can we address deficiencies in past simulations and measurements of bacteriophage T7 to improve models of gene expression? Second, can we design and build refactored surrogates of T7 that are easier to understand and model?

To address deficiencies in past simulations and measurements, I developed a new single-molecule, base-pair-resolved gene expression simulator named Tabasco that can faithfully represent mechanisms thought to govern phage gene expression. I used Tabasco to construct a model of T7 gene expression that encodes our mechanistic understanding. The model displayed significant discrepancies from new system-wide measurements of absolute T7 mRNA levels during infection. I fit transcript-specific degradation rates to match the measured RNA levels and as a result corrected discrepancies in protein synthesis rates that confounded previous models. I also developed and used a fitting procedure to the data that let us evaluate assumptions related to promoter strengths, mRNA degradation, and polymerase interactions.

To construct surrogates of T7 that are easier to understand and model, I began the process of refactoring the T7 genome to construct an organism that is a more direct representation of the models that we build. In other words, instead of making our models evermore detailed to explain wild-type T7, we started to construct new phage that are more direct representations of our models. The goal of our original design, T7.1, was to physically define, separate, and enable unique manipulation of primary genetic elements. To test our initial design, we replaced the left 11,515 bp of the wild-type genome with 12,179 bp of engineered DNA. The resulting chimeric genome encodes a viable bacteriophage that appears to maintain key features of the original while being simpler to model and easier to manipulate. I also present a second generation design, T7.2, that extends the original goals of T7.1 by constructing a more direct physical representation of the T7 model.

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Acknowledgments

To Drew Endy, for his creativeness, telling it like it is, and nurturing a fantastic laboratory atmosphere; also for giving me the freedom to pursue my capricious whims, and reigning me in when needed; but most of all for getting me to share in his quixotic idealism for life and realize it is the only way to be.

To Leon Chan, for his contagious laugh, tireless giving, and love of science; also for being such a great partner in crime on T7.1.

To Heather Keller for her earnestness and keeping the lab and especially me in line all these years; also for her heroic work on the T7 mRNA measurements.

To the Endy Lab for making graduate school so much fun, the selfless atmosphere, late nights talking life and science, and most of all friendship.

To my thesis committee: Doug Lauffenburger, for running a department that I am extremely proud to be a part of and for always looking out for my best interests; Bruce Tidor for honing my interests early on and providing continuing advice and support. Tom Knight for fantastic conversations, guidance, and his love of science; Ian Molineux for his core dumps on T7, letting me stay up later than him at least once (my ego was hurting at that point), and being a guru for all of my work.

To the Sauer Lab, especially Bob Sauer, Chris Hayes, Kathleen McGinness, Sean Moore, and Andreas Martin for teaching me the way of the *coli* and providing endless entertainment with beer, darts, and science.

To beh-one, especially Nate, Maxine, Lisa and Ale for getting me past first-year and friendship through it all.

To the OpenWetWare folk, especially Jason, Ilya, Reshma, Barry, and Austin, for reminding me of the power to act in something you believe in and how much spare time we really have.

To the Harvard folk: Pam Silver and Jeff Way for challenging me to think big, and philosophical conversations on life and career; Becky Ward for getting me to be a better writer, and her interest and support.

To my teachers past and present, for their inspiration.

To Buda folk, for keeping me sane, always having fun, having sick camping trips, and generally providing some of my favorite times and people in Boston.

To my friends and family, for sharing in my highs and for getting me through my lows, Boston winters, and the Bush administration; especially DaveB, Patricia, Richie, Karin, Jason, Samantha, Leon, Sarah, Lisa, Christos, Morgan, Kathleen, Soraya, Aaron, Shani, Annie, DaveL, Max, Chris, Krishna, and Sunil for waxing philosophical and providing love throughout.

Finally, I dedicate this thesis to my mom and dad, for their sacrifices to provide me with opportunities for which I will never be able to repay, all with love, advice, and their support and freedom to not follow it.

Chapter 1. Introduction

Chapter 1.1. Overview

The development of natural biological organisms provides a stunning example of how genetically-identical organisms that have inherent chemical and environmental variation can arrive at complex, deterministic outcomes. The precision and reproducibility of development imply reproducible molecular and cellular events governed by their evolved genome designs. The ability to produce accurate model-based predictions of these molecular and cellular events given arbitrary arrangements of genetic components would allow us to explore why biological systems might be designed in a particular way, and help us build novel, intentional, and useful biological organisms [Carlson R, 2001]. However, making reliable computational model-based predictions remains very difficult [Endy & Brent, 2001]. What remains unclear is what information we are missing, and to what detail do we have to understand it in order to construct predictive molecular models of biological systems [Sharp, 2000].

Our lack of accurate model-based predictions may be due to several reasons. For example, our models may be incomplete, missing details that are important in system function. Also, since models typically are for small subsystems within organisms, the proper boundaries and interfaces between these subsystems and the organism as a whole are poorly defined. We also lack precise experimental data describing the physical state of our system over time, and when we attempt to model the dynamic behavior of the system, make use of limited modeling frameworks that are often poor approximations for the inside of a cell. Not surprisingly, as our models increase in size and complexity, their utility in helping us to predict the behavior that results from novel perturbations is limited.

This thesis is focused on trying to understand the program of gene expression during the development of bacteriophage T7. T7 is particularly well suited as a model system for the development of an entire organism because knowledge of how the phage

functions is thought to be relatively complete. My work focuses on two questions in particular. First, can we address deficiencies in past simulations and measurements of bacteriophage T7 to improve past models of gene expression during development? Second, can we design and build refactored surrogates of T7 that are easier to model and understand?

The remainder of this chapter introduces bacteriophage T7 and past efforts to construct models of T7 development. Chapter 2 introduces a new simulator I developed called Tabasco that allows us to explicitly encode gene expression mechanisms commensurate with our putative understanding of T7 biology. Chapter 3 describes our efforts to build detailed models of T7 gene expression using Tabasco and analyze the models with respect to new system-wide measurements of mRNA levels. In Chapter 4, I present our work on refactoring the T7 genome in order to construct a self-replicating developmental system that is easier to understand. Finally, Chapter 5 describes ongoing extensions of this work and where it stands in relation to my original goals.

Chapter 1.2. Background

Bacteriophage T7 (T7) is an obligate lytic phage that infects *Escherichia coli* [Dunn & Studier, 1983; Studier & Dunn, 1983]. T7 was twice isolated from Ward MacNeal's 'standard anti-coli-phage' mixture [Demerec & Fano, 1945; Delbrück, 1946]. MacNeal's 'mixture' may have been cultured in series – T7 was the only identifiable isolate [Studier, 1979]. One of the two original T7 isolates was reportedly chosen for future use and master cultures of 'wild-type' T7 have been maintained since. Genetics, and then biochemistry, enabled the discovery and characterization of some of the individual elements that participate in T7 development [Molineux, 2005]. Sequencing of the T7 genome revealed additional elements [Dunn & Studier, 1983], not all of which have obvious functions [Chan et al., 2005].

Chapter 1.2.1. Discovering the Parts of T7

T7 is a good model system for learning what fraction of the functional information encoded on the genome of a natural biological system has been discovered, and how much of what is unknown is likely to matter. For example, the T7 protein coding domains were first characterized by the isolation and analysis of randomly generated amber mutants. Nineteen genes were identified by mapping mutants that disrupt T7 DNA synthesis, particle maturation, and lysis [Studier, 1969; Hausmann & Gomez, 1967; Hausmann & LaRue, 1969]. Two additional genes, T7 DNA ligase and protein kinase, were isolated via loss of function and deletion, respectively [Masamune et al., 1971;Ritchie & Malcolm, 1970]; the genetic analysis of ligase and kinase mutants was carried out using mutant host strains that do not support the growth of ligase or kinase defective phage [Studier, 1973a]. As many as 30 T7 proteins were observed by pulsing phage- infected cells with radioactive amino acids [Studier & Maizel, 1969; Studier, 1973b]. Further experiments, such as electrophoretic mobility shifts of amber mutants, provided evidence for as many as 38 T7 proteins [Studier, 1981]. Sequencing of the genome confirmed the previously constructed genetic maps [Dunn & Studier, 1983]. But, analysis of the complete genome sequence also revealed that the set of protein coding domains found via mutagenesis, screening, and mapping was not exhaustive, and that additional unidentified open reading frames occupied most of the remainder of the genome. Some of these unidentified open reading frames can be labeled as putative protein coding domains based on the inferred strengths of adjacent upstream ribosome binding sites. In all, up to 57 genes encoding 60 potential proteins have been found or postulated [Molineux, 2005]. However, only 35 of these 60 proteins have at least one known function. And, of the 25 non-essential proteins, only 12 are conserved across the family of T7-like phage [Molineux, 2005]. Can we ignore these uncharacterized proteins in our models? Should we edit the genome to get rid of them [Chan et al., 2005]?

As a second example of where our knowledge stands, the *E. coli* RNA polymerase promoters on the T7 genome (A0, A1- 3, B, C, and E) were first mapped by in vitro transcription studies [Davis & Hyman, 1970; Minkley & Pribnow, 1973; Golomb &

Chamberlin, 1974a, b; Niles & Condit, 1975; McAllister & McCarron, 1977; Stahl & Chamberlin, 1977; Kassavetis & Chamberlin, 1979; Panayotatos & Wells, 1979] and subsequently confirmed by sequencing [Oakley & Coleman, 1977; Boothroyd & Hayward, 1979; Rosa, 1979; Rosa, 1981a; Rosa, 1981b; Osterman & Coleman; Carter & McAllister, 1981; Dunn & Studier, 1983]. Results of in vitro transcription reactions using T7 genomic DNA as template agreed with the available in vivo transcription data [Studier, 1973a; Summers et al., 1973; McAllister & Wu, 1978; McAllister et al., 1981]. However, the cloning of random sections of the T7 genome into a plasmid that selected for transcription activity from the cloned fragment identified other possible promoters [Studier & Rosenburg, 1981]. Sequence analysis of the cloned sections identified ~10 regions with similarity to known promoters (the identifiable sites are labeled with an S1-S6 in our own annotations); footprinting assays identified two additional promoters (these promoters are labeled F1 and F2 in our own annotations) [Dunn & Studier, 1983; http://web.mit.edu/endy/www/ncbi]. But, any contribution of these putative promoters to wild-type T7 infection is not now defined. As with some of the T7 genes, should we ignore these promoters in our models? Should we delete them from the T7 genome? Is there other information encoded on the wild-type T7 genome that we should include in our models, ignore, or actively remove [Chan et al., 2005]?

Chapter 1.2.2. Putting the Parts Together

The experimentalists who originally discovered much of how T7 works developed the best descriptive, system-level models for T7 infection. Their models were made by integrating knowledge of the individual parts and mechanisms that act during infection, from genome entry to phage particle formation [Studier & Dunn, 1983]. Two features specific to T7 biology made the construction of their system-level models easier. First, compared to other phage, T7 is relatively independent of complex host physiology. For example, T7 encodes phage-specific RNA and DNA polymerases, and mRNA and protein synthesis of host genes is shut down during the first ~6 minutes of T7 infection. Second, RNA polymerase pulls most of the T7 genome into the newly infected cell [Zavriev &

Shemyakin, 1982; Garcia & Molineux, 1995]. Polymerase mediated genome entry is a relatively slow process that results in the direct physical coupling of gene expression dynamics to gene position. For example, a gene cannot be expressed until its coding domain enters the newly infected cell [Chan et al., 2005].

Chapter 1.2.3. Models of Bacteriophage T7 Infection

Building on this work, Drew Endy with John Yin (UW Madison) and Ian Molineux (UT Austin) constructed a model that enables computer-based simulation of T7 development at the 'molecular level' [Endy et al., 1997]. Here, molecular level means that the model captures the expression of individual genes and the interactions among distinct biochemical species such as proteins, mRNA, amino acids, nucleotides, etc. The model starts with a single phage particle bound to a single host cell and follows T7 infection up to intracellular progeny production. The model was built using a best-faith effort at encoding the current scientific understanding of how T7 actually works. Any estimated parameter values were chosen independent of system-level model behavior.

Chapter 1.2.4. How Good is the Model?

Endy's model provides a reasonable estimate of many aspects of gene expression, but several significant discrepancies remained. For example, Figure 1-1 shows the observed and computed rates of protein synthesis during T7 infection. The computed time of first synthesis is correct for most proteins, suggesting that the models for T7 genome entry are good, but that the computed synthesis profiles for a few proteins continue beyond the data (e.g., gp1, the T7 RNA polymerase).

Endy and colleagues then implemented a general form of the T7 simulation that can compute the expected growth dynamics for any permutation of the genetic elements that comprise the wild-type T7 genome [Endy et al., 2000]. Using the new simulator, they computed the expected effects of repositioning each of the essential T7 genes at all

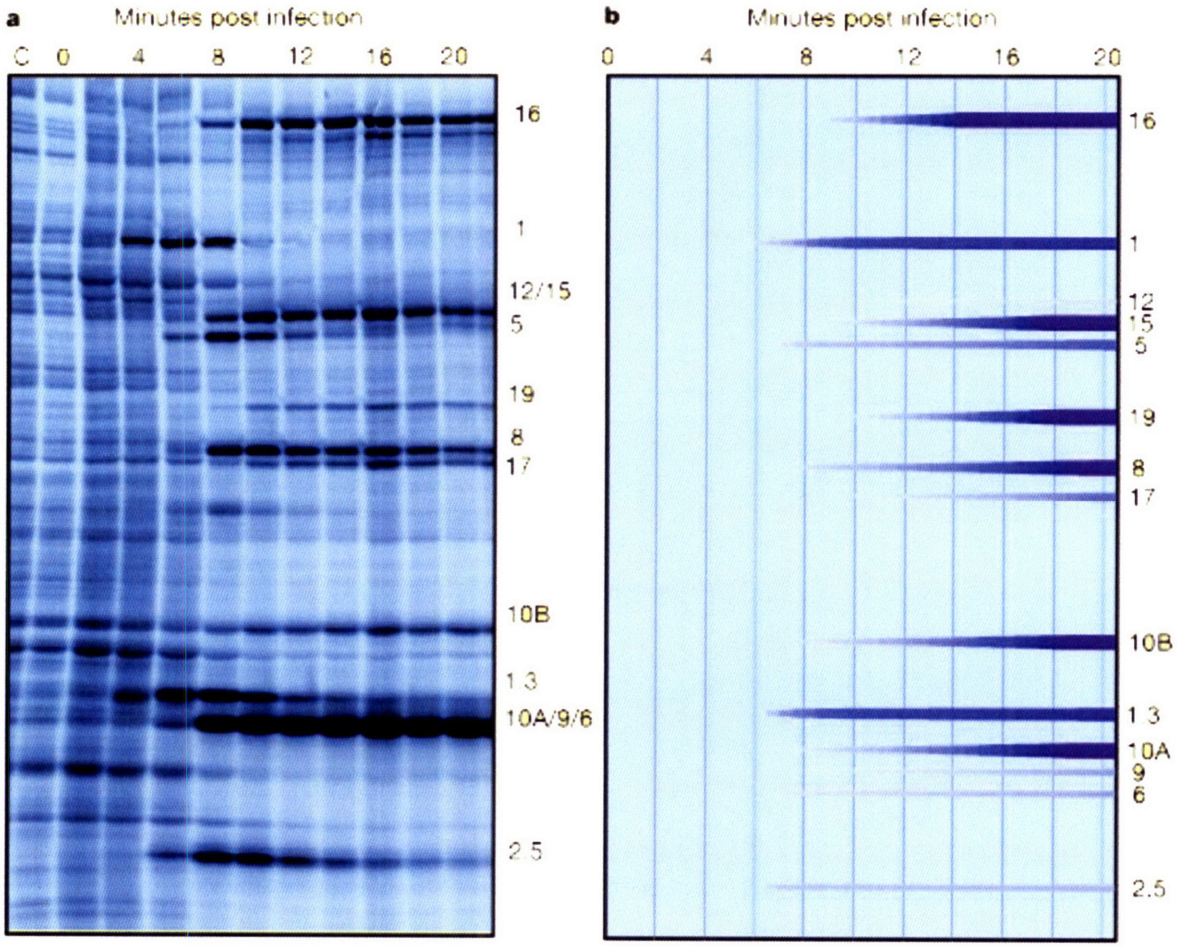


Figure 1-1. Observed and computed rates of phage T7 protein synthesis. (a) Experimental data. 35S pulse-chase protein synthesis gel for wild-type T7 infection. (b) Simulation output. Comparison of observed and computed T7 protein synthesis rates reveals that during an actual phage infection, synthesis of the T7 proteins gp1, gp1.3, gp2.5 and gp5 is down regulated by an unknown mechanism(s) [Endy & Brent, 2001].

possible positions within an array of 72 moveable genetic elements. The simulations predicted that moving gene *1*, encoding the T7 RNA polymerase, immediately downstream of its natural position would produce a mutant phage that grows faster than the wild type under laboratory conditions [Figure 1-2]. The prediction was based on the observation that moving gene *1* to its new position would create a weak T7 autogene [Dubendorff & Studier, 1991], and that modest self-expression of the T7 RNA polymerase would accelerate phage infection. In order to test the prediction, they constructed a series of ectopic gene *1* strains in the lab and, upon characterization, found that all the ectopic phage grew slower than the wild type [Endy et al., 2000].

Disagreement between model-based predictions and the observed system behavior could have occurred for at least three reasons. *First*, past computational approaches to

modeling T7 gene expression could have been inappropriate. For example, studies suggest that noise in gene expression for a system may lead to variation in system behavior [Arkin et al., 1998]. If gene expression noise is significant during T7 development, the creation of the weak autogene in the predicted faster-than-wild-type gene *1* strain, could lead to stochastic differences in phenotype [Thattai & van Oudenaarden, 2001]. For example, one sub-population could produce the autogene behavior, while a second sub-population of cells produces slower-than-wild type infections, and the resulting observed population average growth rate is slower than wild type. *Second*, the resolution of past models may have been insufficient. Past models were constrained by simulation technologies, and thus were unable to explicitly represent mechanisms that are thought to govern gene expression. For example, polymerase-mediated entry of the genome was modeled as a constant rate process, rather than as a result of transcription of the genome. Incorrect representation of such processes can mask problems with the models that would be made clear by explicit simulation. *Third*, models for T7 development could be incomplete or wrong. For example, unknown

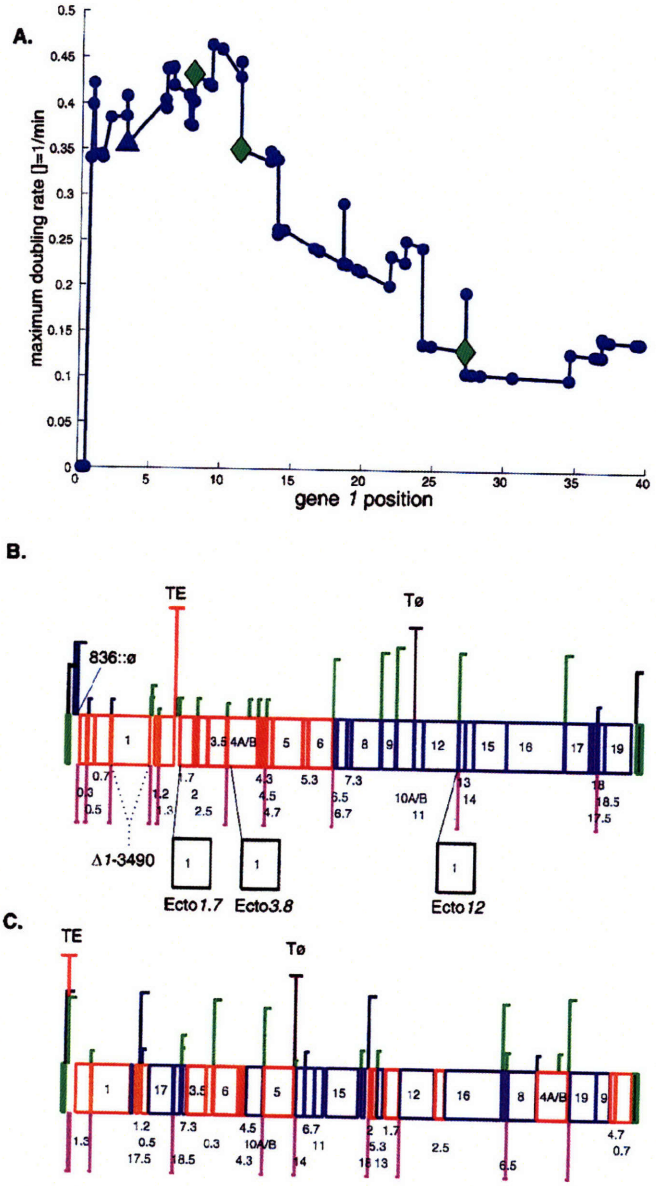


Figure 1-2. (a) Simulation results showing the expected phage T7 growth rate as a function of the position of gene 1, encoding T7 RNA polymerase, on (b) the T7 genome. (c) A mutant T7 genome in which all the T7 genetic elements are permuted [Endy et al., 2000].

genetic elements and regulation mechanisms may play a role in the shutdown of class I and II protein synthesis that the models failed to account for. Using models that do not account for unknown processes could debase any predictions of the models.

In this thesis, I have attempted to address these problems. I developed a new gene expression simulator named Tabasco in order to allow stochastic simulation of the mechanisms thought to govern phage gene expression [§2]. I used Tabasco to make a model of phage gene expression that more directly encodes our understanding of T7 gene expression [§3]. In addition, I coupled this model with comparisons to new system-wide measurements of T7 mRNA levels during development to better evaluate the model's validity. Finally, I began the process of refactoring the T7 genome to construct an organism that is a more direct representation of the models that we build [§4].

Chapter 2. Tabasco

Chapter 2.1. Introduction

Mechanistic models for gene expression are quite detailed and are continuously improving. For example, kinetic studies of promoter initiation and single molecule studies of processive protein movements on DNA give us mechanistic understanding of gene expression at an unprecedented level of detail [for review, Bai et al., 2006]. Those interested in studying the consequences of this understanding on system-level behavior would benefit from the ability to integrate these detailed biophysical models into system-level simulation. Currently available simulation techniques are sufficient to study the expression from a single operon, but become computational infeasible if the system is of much greater complexity [Arkin, 1998].

There are many approaches for encoding systems of biochemical reactions into a mathematical framework. One common approach is to consider the chemical species involved in the reactions as a continuous function of time, for example by expressing the reactions as a set of coupled ordinary differential equations (ODEs) [§2.2.1]. Another approach is to explicitly account for each member of a species, for example by using stochastic simulation algorithms [§2.2.2]. Stochastic simulation algorithms have some theoretical advantages over continuous formulations. For example, the accuracy of ODE simulations can break down when the numbers of any particular reactant in a system are low [Samoilov & Arkin, 2006]. In this situation, an individual reaction event can make a large difference to the likelihood that other reactions will occur, and so the precise order and timing of reaction events matters. Almost any reaction involving a single copy of a gene will have this problem.

Both stochastic and continuous formulations, however, share the problem of the so-called "combinatorial explosion" of individual species and states in a complex system. For example, the spatial and temporal control of the gene *endo16* expression during *S. purpuratu* development is controlled by a 2300 bp sequence [Yuh & Davidson, 1996].

This control sequence contains 33 sites that bind 15 distinct proteins [Yuh et al., 2001]. Simulating this system requires enumerating the possible states of the DNA with proteins bound, which in the case of the regulatory region of *endo16* exceeds 10^{13} distinct species -- far more species than copies of the individual DNA, proteins, and/or complexes in an individual cell. Explicitly enumerating this number of distinct species is currently computationally intractable.

Several groups have addressed the combinatorial explosion problem specifically with respect to protein complex formation [Morton-Firth & Bray 1998; Andrews & Bray, 2004; Lok & Brent 2005]. The simulators designed by these groups (single-molecule simulators) track individual molecules and what they are bound to, so that the only complexes formed at any given time are enumerated, and not all possible states. The rules for moving from one state to another are simplified by defining classes of complexes that undergo the same set of reactions, with reaction rates modulated by the presence or absence of individual components. For example, in the yeast pheromone response pathway, Ste5, a scaffolding protein, can form over 25,000 distinct complexes with other proteins, far exceeding the 5000 complexes estimated to be in the cell at any time [Endy & Brent, 2001; Bardwell et al., 1996]. Using their single-molecule simulator, Moleculizer, Lok and Brent were able to enhance the efficiency of simulating the pathway many fold by in part reducing the number of initial species specified and generating them dynamically as needed [Lok & Brent, 2005].

Current single-molecule simulators deal well with binding and unbinding within complexes. However, they do not deal well with situations in which the elements of the complex are processive (i.e., complexes move along many binding states) or when the number of binding states is large. For example, simulating a single RNA polymerase transcribing a 3,000-base pair gene would require a DNA molecule of over 3,000 different states with enumerated rules for the transitions from one base to the next. In order to simulate detailed models of gene expression, I developed, with Jason Kelly, a single-molecule stochastic simulator, Tabasco, which explicitly deals with reactions specific to gene expression such as the initiation, elongation and termination of RNA transcripts and polypeptide chains and interactions between polymerases [Kosuri S, Kelly, JR, Endy D, in preparation].

Our motivations for creating Tabasco stem from our interest in simulating the dynamics of bacteriophage T7 gene expression during phage infection. Past attempts at constructing models of T7 infection used ODEs to simulate gene expression dynamics, which prevented representation of the details of transcription and entry [Endy et al, 1997, 2001]. For example, the *E. coli* RNA polymerase initiates expression of early T7 genes, including T7 RNA polymerase. T7 RNA polymerase first initiates transcription behind already transcribing *E. coli* RNA polymerase molecules. *E. coli* RNA polymerase has a ~45 nucleotide per second elongation rate; T7 RNA polymerase has a ~250 nucleotide per second elongation rate. What happens when a T7 RNA polymerase molecule catches up to an *E. coli* RNA polymerase? The transition from *E. coli* to T7 RNA polymerase mediated genome entry will impact the timing of expression across the genome; cell-cell variation in the entry transition will limit the precision by which T7 can control infection. Using Tabasco, we are able to explore the dynamics of gene expression during T7 development at single-base resolution. The increased resolution of these computational models allows for direct evaluation of assumptions concerning polymerase interactions, transcriptional coupling to genome entry, and stochastic fluctuations on T7 development.

Chapter 2.2. Background

Chapter 2.2.1. Mathematical Models of Gene Expression

The history of mathematical and biophysical models of gene expression began at the very start of our understanding of the molecular nature of genetic information [Luria & Delbrück, 1943]. I will focus this section primarily on the development of biophysical models of the transcriptional control of prokaryotic gene expression through experiments and the parallel development of mathematical models.

In 1961, Francois Jacob and Jacques Monod showed through a series of experiments and logical formulations that the control of β-galactosidase expression is dependant upon negative regulation by lac repressor (Jacob & Monod, 1961). Furthermore they hypothesized that these interactions depend upon a secondary

messenger, which acted as a temporary intermediary between the genetic code and protein synthesis by the ribosomes. Sydney Brenner, Monod, and Matthew Meselson experimentally verified the existence of this messenger RNA (mRNA) soon afterwards, which solidified the "central dogma" of biology [Brenner et al., 1961]. Work on the lac system has since continued, leading to such discoveries as the notion of the operon as a module of control for genetic systems, positive control of gene expression, and technologies such as DNA sequencing [reviewed in Muller-Hill, 1996].

Soon afterwards, Stuart Kauffman constructed some of the earliest mathematical models of gene expression in order to study how large-scale biological systems might be organized [Kauffman, 1969]. Kauffman treated the expression state of each gene as a binary variable (on/off), whose output is dependent upon some Boolean function of its inputs. Others, such as René Thomas, formalized these studies and extended them to include delays that represented times between initial signal and output protein production [Thomas, 1973; Thomas et. al., 1976]. Also around this time, Michael Savageau began to study feedback control in genetic systems using ordinary differential equations [Savageau, 1974; 1975; §2.2.2]. Savageau's approach was to model gene expression as a system of chemical species governed by synthesis and degradation terms that were dependent upon power-law relationships with other species. These studies formed the basis for the mathematical modeling of gene expression using coupled sets of differential equations.

Alongside these mathematical studies, experiments attempting to understand the biophysical process of transcription initiation were ongoing. For example, two regions, the Pribnow box and the –35 region, located 10 and 35 bases from the transcription start respectively, were found to be conserved in *E. coli* promoter regions and thought to function as the DNA binding site for RNA polymerase [Pribnow, 1975; Gilbert, 1976]. This information was brought together into what was called the "bipartite model" for promoter initiation. In this model, sequence specific binding was in fast equilibrium relative to the slower process of DNA open complex formation, which together determined the initiation rate of the polymerase [McClure, 1982]. In addition, details were uncovered on the abortive initiation of polymerases, which showed that polymerase

seem to start, abort, and then restart a number of times before the formation of a productive elongation complex [Johnston & McClure, 1976; Cech et. al., 1980].

Based on these new biophysical models, a new class of mathematical models was emerging. Shea and Ackers' work on modeling the gene regulation of the bacteriophage lambda O_R control region created a framework based on statistical mechanics [Ackers et. al., 1982; Shea & Ackers, 1985]. They postulated that since the binding of operators to DNA was very fast when compared to downstream processes such as transcription initiation, a probabilistic model of binding could be created based on proportionality of the equilibrium binding constants of proteins to the DNA.

The importance of the Shea-Ackers' model was not fully appreciated until work on understanding the role of random fluctuations in biochemical systems began. Though the phenomenon of cell-cell variation in the bacterial response to chemical stimuli has been apparent for some time, little progress was made in trying to mathematically model it [Luria & Delbrück, 1943; Chock & Stadtman, 1977; Goldbeter & Koshland, 1981]. However, a number of improvements allowed for the development of stochastic simulations that could probe such behavior. First, the development of exact stochastic simulation algorithms (SSAs), when coupled to increases in computational power, allowed for the efficient simulation of random fluctuations in larger chemical systems [Gillespie, 1976, 1977; §2.2.2]. In addition, the Shea-Ackers model allowed for the treatment of fast DNA-binding reactions as being in equilibrium, easing the computational burden. Adam Arkin and Harley McAdams showed that stochastic effects can be successfully modeled using these new simulation tools, and that cell-cell variation due to uncertainty in the timing of individual reaction events might qualitatively impact developmental pathways [McAdams & Arkin, 1997; Arkin et. al., 1998]. Several groups have subsequently experimentally and theoretically explored the effects of stochastic variation on gene expression [Thattai & van Oudenaarden, 2001; Ozbdak et al., 2002; Elowitz et al., 2002; Paulsson, 2004; Rosenfeld et al., 2005; Pedraza et al., 2005; Kaern et al., 2005; Mettetal et al., 2006].

Most mathematical models of gene expression still lag behind our experimental understanding of gene expression. For example *in vitro* single-molecule studies revealed that pauses and arrests of *E. coli* RNA polymerase transcribing along DNA occur often,

and that pausing is a step in the route towards termination [Forde et. al., 2002; Adelman et. al., 2002; Skinner et al., 2004]. However, such mechanisms are difficult to account for with current simulators. In another example, interactions between transcribing polymerases on the genome are known to affect gene expression [Adhya & Gottesman, 1982; Horowitz & Platt, 1982; Nomura et al., 1985; Epshtein et al., 2003]. Again, simulating interaction of transcribing polymerases is difficult. In both cases, the near base-pair resolution needed to analyze these models is difficult because of the large number of species that need to be enumerated in such a detailed simulation.

Chapter 2.2.2. Stochastic Simulation

Simulating the time course of a set of coupled chemical reactions is often carried out using ordinary differential equations. This approach to representing biological systems approximates all species as continuous functions of time. However, in some cases, the continuous approximation breaks down. When individual reaction events cause large changes in the probability of future reactions, the precise order and timing of reaction events matters. Variation in the timing of individual reactions due to thermal fluctuations can produce variation in system behavior that is not accounted for in deterministic formulations. For example, variation can be large when there are low numbers of any individual reactants, as in the case of single-copy genomic DNA. Stochastic formulations, on the other hand, are able to account for this variation by treating all species as discrete variables and reaction rates as probabilities per unit time (i.e., propensities). Solving these stochastic formulations analytically is very difficult and practically impossible except for the most simple of systems [e.g., Thattai & van Oudenaarden, 2001]. Thus, the most common approach is to use computational methods developed by Dan Gillespie in the 1970's to approximate solutions [Gillespie, 1976, 1977].

Gillespie's stochastic simulation algorithm (SSA) allows for exact simulation of a system of elementary chemical reactions [Gillespie, 1976]. For each reaction, the SSA generates a tentative execution time by mapping a randomly generated number to the reaction time's probability density function (PDF). In the case of an elementary chemical

reaction, the reaction time's PDF is defined as an exponential distribution with characteristic time proportional to the inverse of the reaction's propensity. A reaction propensity is related to the macroscopic rate constant of the reaction and is also dependent upon the number of reactant molecules and cell volume. In a system of reactions, the algorithm first executes the reaction with the earliest tentative reaction time, and updates the copy numbers of the reactant and product species. The overall simulation time is then incremented by the tentative reaction time, and the propensities of all the reactions are recalculated. This process is repeated and, in this manner, one potential time course of the system is generated. When averaged over many runs, the distribution created is equivalent to the analytical solution obtained through the chemical master equation, which describes all the probabilistic states of the system [Gillespie, 1977].

Subsequently, there have been improvements to Gillespie's SSA that help increase the computational efficiency, such as Gibson and Bruck's accelerations to the Gillespie's SSA in the Next Reaction Method (NRM) [Gibson & Bruck, 2000]. First, instead of recalculating all reaction propensities and times, the NRM efficiently transformed them from one cycle to the next. Second, the NRM created a data structure, called the reaction graph, in order to only update those reactions whose substrates' copy numbers have changed after any particular reaction has occurred. Third, more efficient data structures, priority queues, were used to search for the first reaction. Finally, Gibson and coworkers outlined the use of the NRM to model a series of reactions that have identical exponential propensities (e.g., polymerization) as a single reaction described by the gamma distribution.

Chapter 2.3. Results

Chapter 2.3.1. Features and Structure of Tabasco

Tabasco is a stochastic simulator that tracks individual molecules of DNA and associated proteins at single base-pair resolution. By defining logical rules of

transcription and translation that govern initiation, elongation, termination, and interactions of polymerases, ribosomes, and proteins at the start of simulation, Tabasco is able to dynamically update the state of the system and generate the appropriate reactions [Fig 2-1]. For example, if a polymerase transcribes over another protein's DNA-binding site, the simulator makes the site unavailable for binding other proteins until the polymerase is no longer overlapping the binding site. Tabasco makes use of a Gibson-accelerated Gillespie stochastic simulation algorithm (SSA) to compute the reaction event timing and the resultant time-evolution of the genetic system [Gibson & Bruck, 2000].

Tabasco's structure confers a number of advantages [Figure 2-2; Methods]. First, tracking the state of individual molecules on DNA eliminates the need to enumerate all the possible states of polymerases and proteins associated with the DNA. Second, protein-protein interactions that may occur on DNA, such as collisions between different

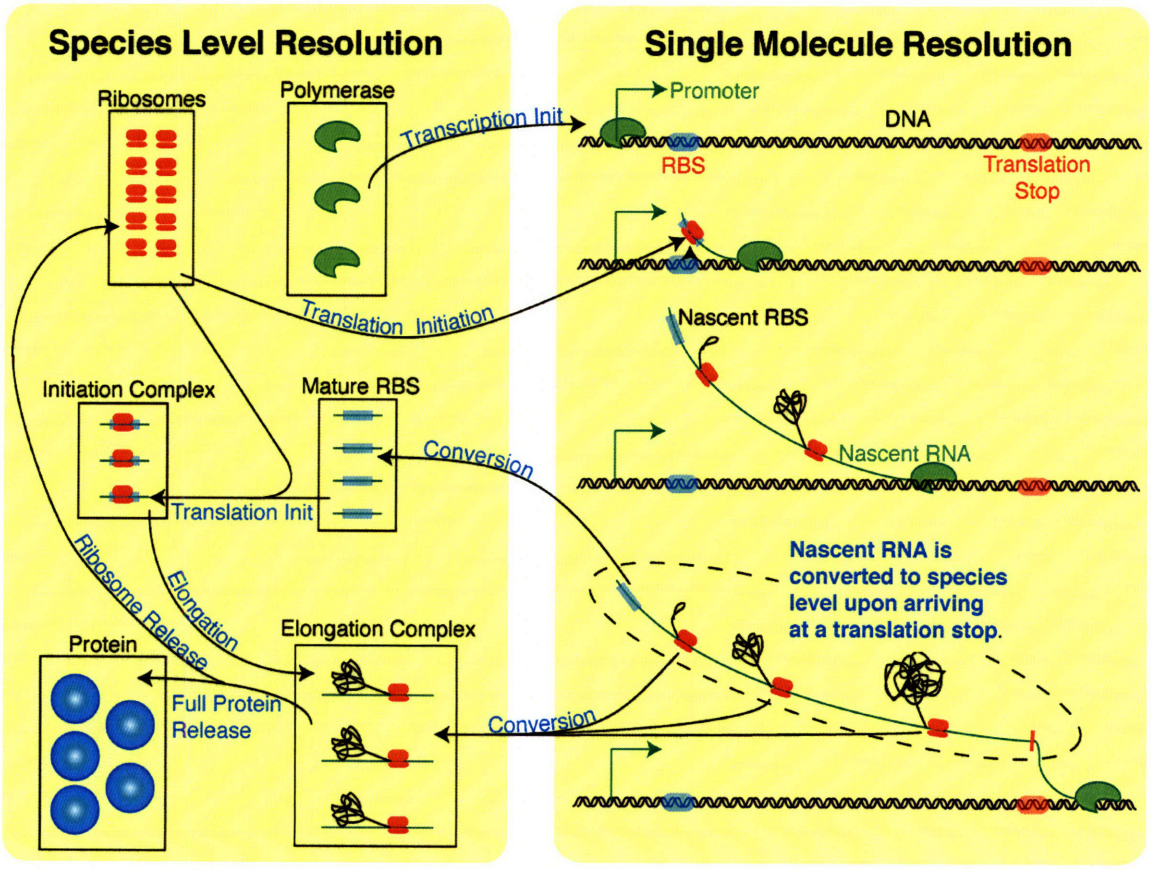


Figure 2-1. Structure of Tabasco Simulator. A simplified flow diagram of gene expression shows how Tabasco transitions from tracking individual molecules (Single Molecule Resolution) to grouping them into species (Species Level Resolution). The arrows represent reactions that can occur (process labeled in blue). See §2.5.2 for an explanation of

polymerases or between polymerases and other proteins, can be accounted for and simulated based on explicitly defined rules. Third, Tabasco allows us to consider and integrate a very large number of factors that may influence the rate of RNA polymerization for any particular gene, such as the binding of multiple transcription factors or the contribution of RNA polymerases that initiated transcription at a promoter connected to an upstream gene. Fourth, Tabasco can be used to visualize the location and progress of the polymerases transcribing DNA. Two advantages of Tabasco that are particularly relevant to T7 are that the simulator allows one to define multiple polymerases with different characteristics (such as transcription speed) and that Tabasco tracks the time course of the entry of the T7 genome, allowing us to see how the stage of entry changes the dynamics of gene expression [see movie at http://openwetware.org/wiki/Tabasco; §3].

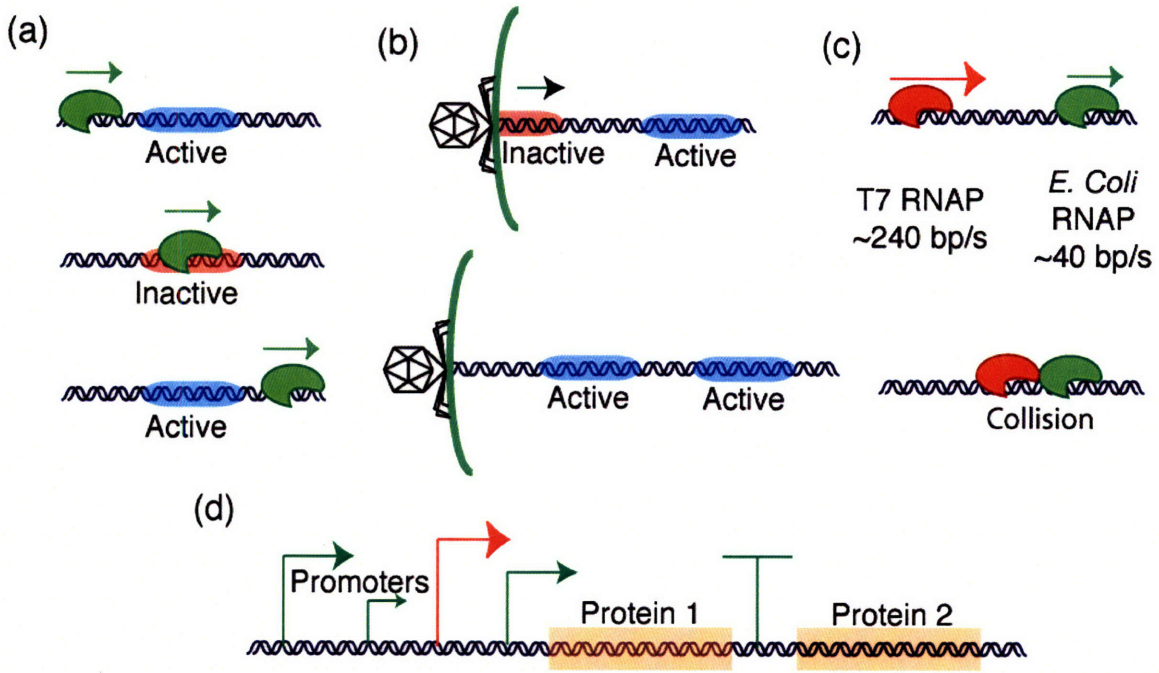


Figure 2-2. Advantages of Single Molecule Resolution. Tracking individual molecules of DNA at a base-pair resolution allows for enumeration of mechanisms that were previously not examinable through simulation. For example, when a traversing polymerase passes over a genetic element such as a protein-DNA binding site, the binding site is inactivated and prevented from binding a protein until the polymerase completely passes over (a). Activation of genetic elements by entry of DNA into the cell (b) and inter-polymerase interactions (c) can be directly simulated as well. Finally, in complex genetic environments (d), a priori transcription levels for each protein need not be calculated, for they are generated implicitly (Promoter arrows with different sizes and colors represent different activities and polymerase specificities, respectively).

While treating DNA with single-molecule and single base-pair resolution provides a number of advantages, treating RNA and protein species at the same resolution is both unnecessary and computationally expensive because of the large number of RNA molecules compared with DNA. To increase computational efficiency Tabasco transitions from single-molecule simulation, where individual molecules and their states are tracked, to species-level simulation for RNA and protein molecules, akin to traditional SSAs. Only those RNA molecules that are still attached to transcribing RNA polymerases are treated as single molecules. As each coding domain on an RNA molecule is completed, it becomes part of the species-level simulation. In essence, each of the coding domains is treated separately as a species, and ribosomes that initiate translation on a coding domain are assumed not to interfere with one another [§2.4]. Translation is treated as a series of single amino acid polymerization steps, with the number of steps depending on the length of the coding domain. This gives a more accurate distribution of times for protein production than treating the whole elongation process as a single step. Based on the work of Gibson and Bruck, we model these steps in aggregate using the gamma distribution [Gibson & Bruck, 2000; Figure 2-3]. Protein interactions are all treated at the species-level.

Chapter 2.3.2. Simple Gene Expression Models

As an initial test, Tabasco was used to simulate expression of a 1000 amino acid protein and compared against results from a standard species-level SSA [Figure 2-4]. The structure of the Gillespie-based species-level SSA is comparable to the Tabasco structure, except that processive movements on the DNA are treated as a lumped, single-step reaction sampled from an exponential distribution [Figure 2-1; Figure 2-6]. Care was taken to make the simulations as close as possible for comparison; some differences were unavoidable, but they were insignificant [Table 2-1; §2.5.5.2; §7.3.1]. In the steady state, both simulators produce similar results. However, a difference arises in the pre-steady state kinetics of the system. The species-level simulation first starts producing protein by ~30 seconds. This is unrealistic; RNA polymerase transcribes at an average

rate of 40 bp per second, so production of a 3,000 bases of messenger RNA should take ~75 seconds. Even if a ribosome follows directly behind the RNA polymerase, protein production should not take less time than RNA production. Tabasco produces protein only after a more realistic ~100 seconds.

The discrepancy in time to the first protein production between the species-level and Tabasco simulators occurs for two reasons [Gibson & Bruck, 2000]. First, since the RNA polymerase and ribosome elongation steps in the species-level simulation are treated as single exponential elementary reactions, 63% of the reactions happen earlier than the average reaction time. The second reason, which is more substantial, is that SSAs assume that the reactions are Markov, i.e., reaction timings do not depend on

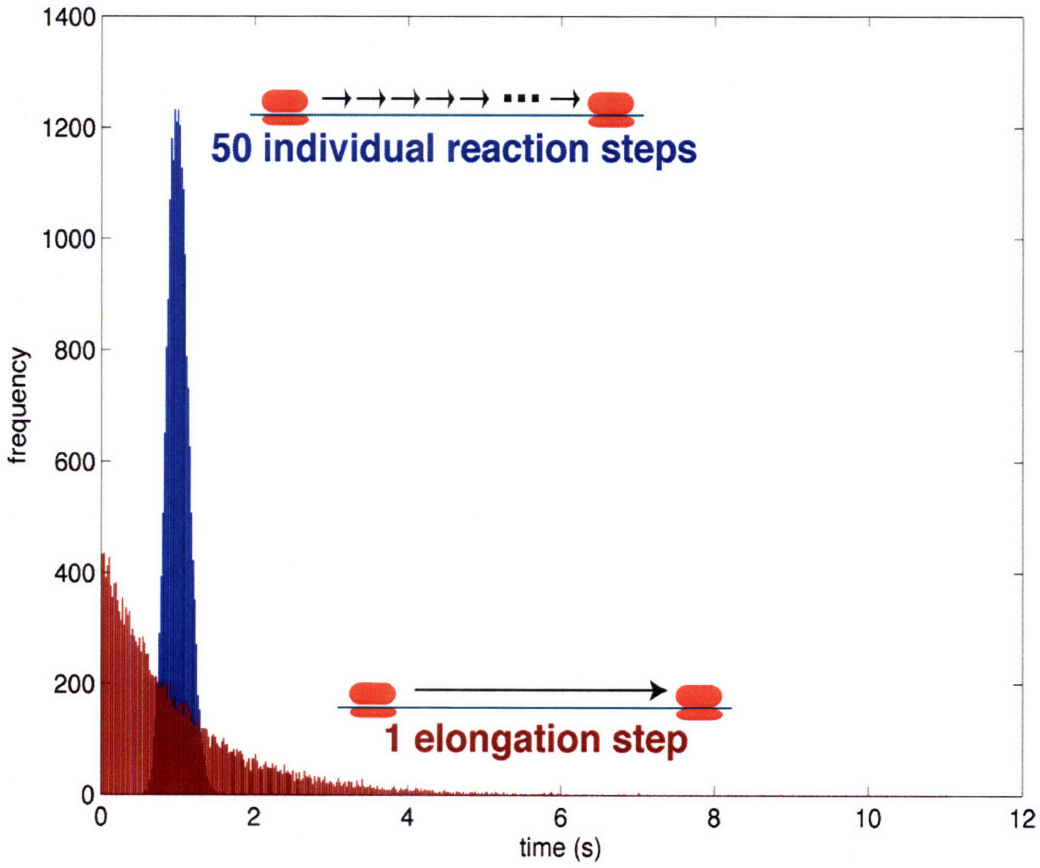


Figure 2-3. Differences in single-step and multi-step elongation processes. Treating elongation of polymerase on DNA along 50 bases as a one step elongation process versus a chain of individual reaction steps gives the same average elongation time, but has marked differences in the distribution of those elongation times. The process shown here is either a single 1 second elongation step (red) or fifty 1/50 second elongation steps (blue). Each histogram is the distribution of resulting times from 20,000 independent simulations.

history. In an SSA that treats elongation as a single step, a ribosome has a chance of entering the elongating state, and a chance of leaving it; the greater the number of ribosomes that have entered elongation, the greater the chance that a protein will be completed. Thus, there is a chance that a protein will be completed in a very short time after elongation begins; especially if many ribosomes enter an elongating state before

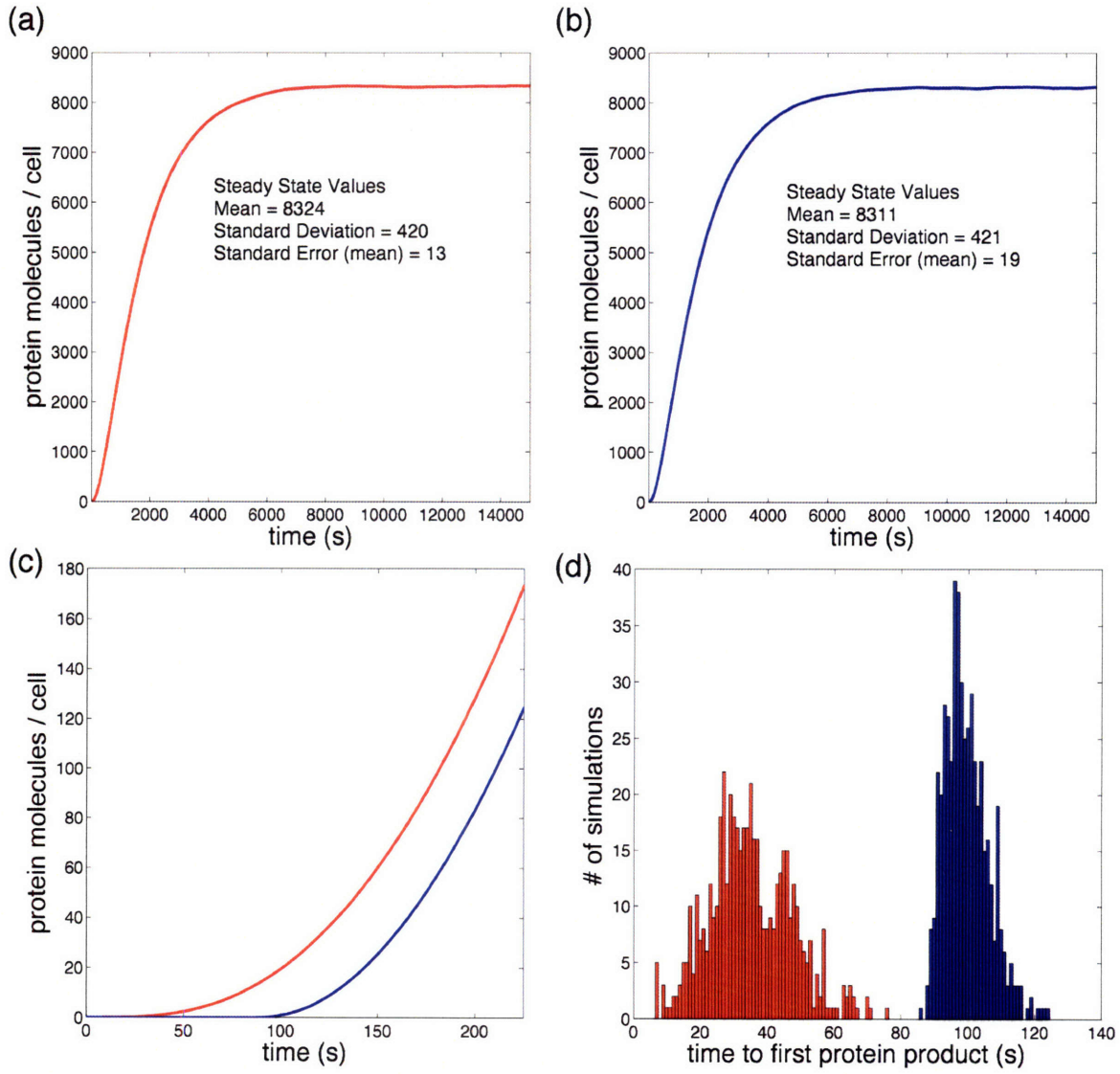


Figure 2-4. Comparison of Tabasco to species-level simulations for a simple gene. I simulated expression of a 1000 amino acid protein using Tabasco (blue) and a standard species level simulator (red), where the processes of elongation (transcription and translation) were lumped into single steps. While the steady-state averages are quite similar (a-b), the pre-steady state kinetics are quite different (c). The distribution of times to produce the first protein for all the simulations is shown in (d). The species-level simulator generates unrealistic times to produce a protein of this size; elongation of the polymerase alone should take ~75 seconds (see text). The data shown are averages of 500 simulations.

ever leaving the elongating state. The fact that Tabasco treats each elongation reaction as a series of elongation steps alleviates both of these problems. These improvements provide a more realistic timing for simulation of pre-steady state kinetics of protein production that are important during dynamic phenomena such as signal transduction or embryonic development.

Chapter 2.3.3. Polymerase Interactions

The specific system I have chosen to study using Tabasco is bacteriophage T7. During bacteriophage T7 infection, the *E. coli* RNA polymerase initiates expression of early T7 proteins, including T7 RNA polymerase [Studier & Dunn, 1983]. T7 RNA polymerase first initiates transcription behind already transcribing *E. coli* RNA polymerase molecules. At 37°C, *E. coli* RNA polymerase transcribes at ~45 bp per second; T7 RNA polymerase transcribes at ~250 bp per second [Garcia & Molineux, 1995]. Thus it is almost inevitable that the T7 RNA polymerase will collide with *E. coli* RNA polymerases ahead of it.

Tracking the DNA at a single-molecule level allows us to simulate how protein output might change as a result of the assumptions made on the outcome of RNA polymerase collisions [Adhya & Gottesman, 1982; Horowitz & Platt, 1982; Nomura et al., 1985; Epshtein et al., 2003]. Figure 2-5 shows the results of simulation of a simple genetic system where two different polymerases transcribe the same piece of DNA, mimicking the layout of the T7 genetic architecture. The simulations describe three possible outcomes of collision: (1) the downstream polymerase in the collision terminates, (2) the upstream polymerase terminates, or (3) the upstream polymerase follows at the speed of the downstream polymerase. Each assumption leads to different levels of steady state protein production. For example, in cases where the upstream polymerase terminates upon the collision of polymerases, most of the T7 polymerases will prematurely terminate because they will catch up with a slower, previously transcribing host polymerase. In this case, the resulting gene expression levels are similar to the situation where there is no T7 polymerase at all.

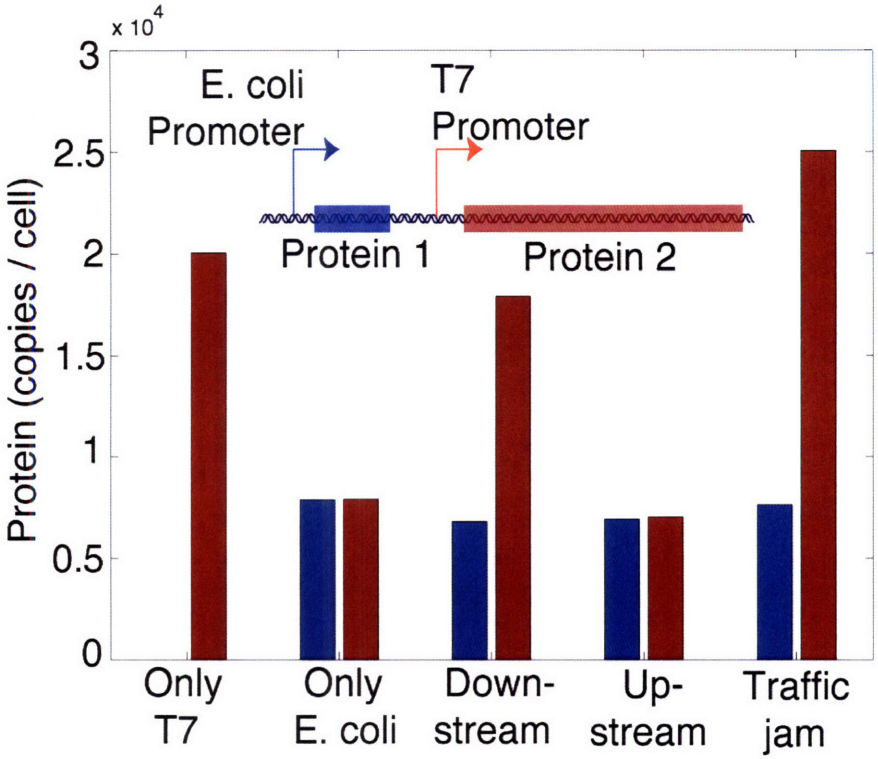


Figure 2-5. Simulating models for inter-polymerase interactions. Average steady-state protein levels of simulations of the DNA molecule with promoters for different polymerases (T7 and *E. coli* RNA polymerases) that are expected to interact on the DNA as they transcribe. The T7 RNA polymerase is ~5 times faster than the *E. coli* RNA polymerase. Shown above are the results of simulations where different assumptions are used as to the nature of the interaction that occurs between the polymerases upon collision. The 'Downstream falloff' model specifies that the downstream polymerase upon a collision between two transcribing polymerases will fall terminate transcription and release from the DNA. The 'Upstream falloff' model describes the opposite situation when the upstream polymerase falls off. Finally, the 'Traffic jam' model is when the upstream polymerase follows the downstream polymerase at the speed of the downstream polymerase. The 'Only T7' and 'Only E. coli' are controls that show expression without *E. coli* and T7 RNA polymerase, respectively.

Chapter 2.3.4. Genome-scale Models – Bacteriophage T7

In order to test the effectiveness of using Tabasco on larger, more biologically realistic systems, I simulated gene expression during bacteriophage T7 infection. The 39,937 base-pair genome, as represented by the simulation, contains 52 coding domains, 22 host and phage promoters, two transcriptional terminators, and three distinct transcriptional feedback loops for each infecting phage molecule [Dunn & Studier, 1983].

Because there are relatively few transcriptional terminators and many promoters, the rate of transcription of individual genes in this system depends on the rates of transcription of the upstream genes, as well as the presence of different polymerases. In the case of T7, there is a further complication in that not all genes are available for transcription at once, since the entry of the T7 genome is a relatively slow process.

Tracking the development of T7 from entry to the end of gene expression is thus a good test for simulation of genomic level models in Tabasco. Using realistic parameter sets, I simulated the simultaneous infection of three phage into a single cell [§2.5.5.3]. Jason Kelly and I developed a method to visualize the results of these simulations that allows graphical display of Tabasco output [see http://openwetware.org/wiki/Tabasco].

Simulation of a full scale genomic model of T7 gene expression during infection on a single AMD Athlon MP 2100+ 1.8 GHz processor takes on the between 1-2 hours for 30 minutes of simulated infection time. For the first time, we are able to simulate gene expression at single base resolution for all gene expression in a particular organism during development. In particular, we can explore specific hypothesis of polymerase interactions, stochastic gene expression, and coupling of entry and transcription.

Chapter 2.4. Discussion

I designed and built Tabasco in response to the inability of currently available simulation tools to represent biophysical mechanisms at a level of resolution commensurate with our understanding. Tabasco allows simulation of T7 genome scale models of gene expression at single base-pair resolution. Previous tools required either modeling smaller systems at increased resolution, or simplifications and assumptions of the biophysical models that result in less accuracy. For example, Tabasco's implemention of a Gibson-accelerated Gillespie SSA allowed us to bypass standard simplifications that are often made for promoter binding and initiation [Shea & Ackers, 1985]. I have shown here that the increased resolution provides more accurate pre-steady state kinetics of gene expression, gives us the ability to test models of polymerase and protein interactions on the DNA, and allows us to fully simulate the dynamics of gene expression during

development of bacteriophage T7.

Executables, source code, documentation, and usage notes for Tabasco are freely available, and should facilitate future extensions on the current design [http://openwetware.org/wiki/Tabasco]. While requiring significant processing power, Tabasco is computationally tractable in regimes where the effects of perturbations are highest; i.e., when the number of DNA molecules in the system is low. The simulator should be a good base to further study interactions on DNA that lead to transcription and translation. For example, Tabasco should provide a platform to explicitly simulate hypotheses how many transcription factors can interact to direct eukaryotic gene expression, such as in control of *endo16* expression [Yuh et al., 2001]

There are many other tools for stochastic simulation available; Tabasco specifically enables simulation of high-resolution models of gene expression. Other single molecule simulation tools, such as StochSim and Moleculizer, are available and primarily focus on binding and activation of protein complexes in signaling. In addition, others have been working on increasing the efficiency of the SSA without significantly sacrificing accuracy [Rao & Arkin, 2003; Gillespie & Petzold, 2003; Cao et al., 2005]. Finally, I am helping to construct a simulator that uses rule-based logic like Tabasco to simulate proteoglycan formation and modification (§5.1). As our models for system level behavior become more encompassing, future work will require combining the efficiencies gained from these individual simulators to create more general tools for high-resolution models of system behavior.

Chapter 2.5. Methods

Chapter 2.5.1. Overall Structure and Gillespie Integration Simulator Structure

Tabasco implements a modified version of the Next Reaction Method (NRM) [§2.2.2; Gibson & Bruck, 2000]. Briefly, at the start of the NRM, the reactions are

defined and their tentative time of next execution is calculated and stored within a priority queue containing all the defined reactions. The priority queue sorts these reactions efficiently to allow quick searches for the minimum tentative time. The reaction with the next tentative reaction time relative to the current simulation time is executed, any tentative reaction times that are affected by the current reaction's execution are updated, and the priority queue structure is reordered to reflect the new times. The process is repeated to compute the time evolution of the system.

The NRM uses a dependency graph to determine which reactions are affected by any particular reaction's execution. In the NRM, the dependency graph is calculated a priori, and remains unchanged throughout the simulation. However, since Tabasco creates reactions and complexes at the single-molecule level dynamically during simulation, a static dependency graph and priority queue will not work. In order to solve this problem, Tabasco contains two specialized reactions per DNA molecule within the priority queue and dependency graph that are used to track a set of dynamically generated reactions. These reactions are placeholders for all the dynamically generated transcriptional and translational events that occur at the single-molecule level. The placeholder reactions have tentative reaction times that are equal to the minimum tentative reaction time of set of dynamically generated reactions the placeholder is tracking. These placeholder reactions are described below [§2.4.2].

Chapter 2.5.2. Transcription and Translation

There are two specialized placeholder reactions within the priority queue in the NRM that are used to track DNA-protein complexes and RNA-ribosome complexes at the single-molecule level. The DNA-protein complex reaction tracks the position and state of polymerases, or other proteins, as they traverse the DNA. The RNA-ribosome complex reaction tracks the state of those ribosomes elongating along mRNAs that are still being transcribed by RNA polymerases. Here state refers to whether the polymerase or ribosome is just bound, initiating, or elongating, as well as the time they will next perform an action such as termination or elongation. All the complexes, and the events

that they may generate, are stored in a different priority queue contained within these specialized reactions. The minimum tentative reaction time for any of the complexes to change state or position is used as the tentative time for the entire placeholder reaction in the NRM.

It is within these specialized entries that the logic of transcription and translation is encoded. In the simulation, polymerases bind free promoters to form a protein-DNA complexes, which are stored within the specialized NRM entry. These bound complexes can then either form an initiation complex or fall off the DNA. Once an initiation complex is formed, the polymerase can undergo abortive initiation steps, or it can begin transcription. The promoter is not freed until the footprint of the polymerase has left the entire promoter region. If two elongating polymerases collide, they act according to the polymerase interaction model chosen at the start of simulation. Upon arriving at a transcriptional terminator, a polymerase will either continue transcribing or terminate depending on a stochastic decision that is weighted by the termination efficiency. Polymerases that reach the end of a particular piece of DNA also terminate transcription. In addition, transcribing polymerases that are on functional protein binding sites such as other promoters, block binding of the site by other proteins until the polymerase footprint clears the site.

Transcribing RNA polymerases produce mRNA, which must be tracked by Tabasco. I used two classes to represent ribosome binding sites (RBSs) on the genome, Nascent RBSs and Mature RBSs, to differentiate when mRNA is tracked at the single-molecule level or the species-level, respectively. The Nascent and Mature RBSs both represent a single RBS on the DNA, and the two are distinguished here in order to deal with the computational structure of Tabasco. When an RNA polymerase first transcribes an RBS, a Nascent RBS is created. If, for example, a RNA polymerase prematurely terminates before reaching the translation stop of the Nascent RBS, the mRNA and the ribosomes translating it must be deleted from the simulation as well. Thus, while the coding sequence is still being transcribed, we must treat all translation events at the single-molecule level as well. Once the polymerase arrives at the corresponding translation termination site, the Nascent RBS is converted to a Mature RBS which is tracked at the species-level.

Translation occurs in three steps. First, ribosomes can bind either Nascent or Mature RBSs. There is an initial reaction to form the Initiation Complex. Second, these initiation complexes are converted to Elongation Complexes and an RBS (Nascent or Mature) at a rate dependent upon the speed of the ribosome and the length the ribosome must travel to clear the ribosome binding site. This reaction is treated as a single step, however the distribution of times are chosen from the gamma distribution, in order to represent a series of exponential reactions. Third, the Elongation Complex then forms a finished protein and a free ribosome at a rate proportional to the remaining length of the open reading frame. This reaction also is represented using the gamma distribution.

Chapter 2.5.3. DNA entry

In order to simulate mechanisms of bacteriophage T7 entry into the cell, we used a two-step DNA entry model. First, DNA can enter at a constant rate, up to a certain site, which is meant to represent a molecular motor based entry of phage DNA. Second, RNA polymerases have been implicated as molecular motors that drive phage DNA entry [Zavriev & Shemyakin, 1982; Garcia & Molineux, 1995]. In Tabasco, RNA polymerases that reach the end of the DNA that has not yet entered the cell cause DNA internalization at the rate of polymerization. These mechanisms were added to Tabasco, because they are thought to govern bacteriophage T7 entry.

Chapter 2.5.4. Simulation, Data Output, Visualization, Code

Tabasco is written in Java® 1.4. The input file to the simulator is an XML file that describes and parameterizes the relevant genetic elements, initial conditions, and any other reactions that occur. The visualization is created by producing images that are then merged using Quicktime® to create a movie. The source code, executables, along with documentation and instructions for use, are available online at http://openwetware.org/wiki/Tabasco

Chapter 2.5.5. Parameterization

All constants used are provided for completeness. For a discussion on the sources and/or derivations of the constants used, see Chapter 3.

Chapter 2.5.5.1. Gamma versus Exponential distribution

The distribution of expected times for a reaction to occur in a stochastic simulator depends on the underlying model [Figure 2-3]. Elementary chemical reactions will follow an exponential distribution in arrival times. However, this is not true of non-elementary reactions. Treating an imaginary elongation process as one step versus 50 individual steps has significant consequences. To obtain the distribution time for the two cases, I use uniformly-distributed pseudorandom numbers and transform them into exponential- or gamma-distributed random numbers. The exponential distribution is used for the single step representation. Exponentially distributed numbers are calculated by simply taking the negative natural log of a uniformly-distributed pseudorandom number. A series of exponentially distributed arrival times, as in the case of the multi-step elongation process, is given exactly by the gamma distribution. Gamma distributed numbers are calculated from uniformly-distributed pseudorandom numbers by an implementation of the rejection method [Press et al., 1992]. Pseudorandom numbers are generated from Java's implementation (java.util.Random) of a linear congruential pseudorandom number generator with a 48-bit seed [Knuth, 1997].

Chapter 2.5.5.2. Simple Gene Expression model

I simulated two models of a promoter driving expression of a coding domain. The first model, termed single-molecule simulation, used the described Tabasco simulator to account for each reaction step during transcription and translation. The model uses the

schemes shown in Figures 2-1 and 2-6a. The second model, termed the species-level simulation, treats transcriptional and translational elongation as a single step as shown in Figure 2-6b. The main parameters used in both models are shown in Table 2-1. Constants were adjusted slightly to account for small differences in model structure to give equal steady state values of mRNA and protein levels. Finally, the input files for the simulations can be found in the Appendix [§7.3.1].

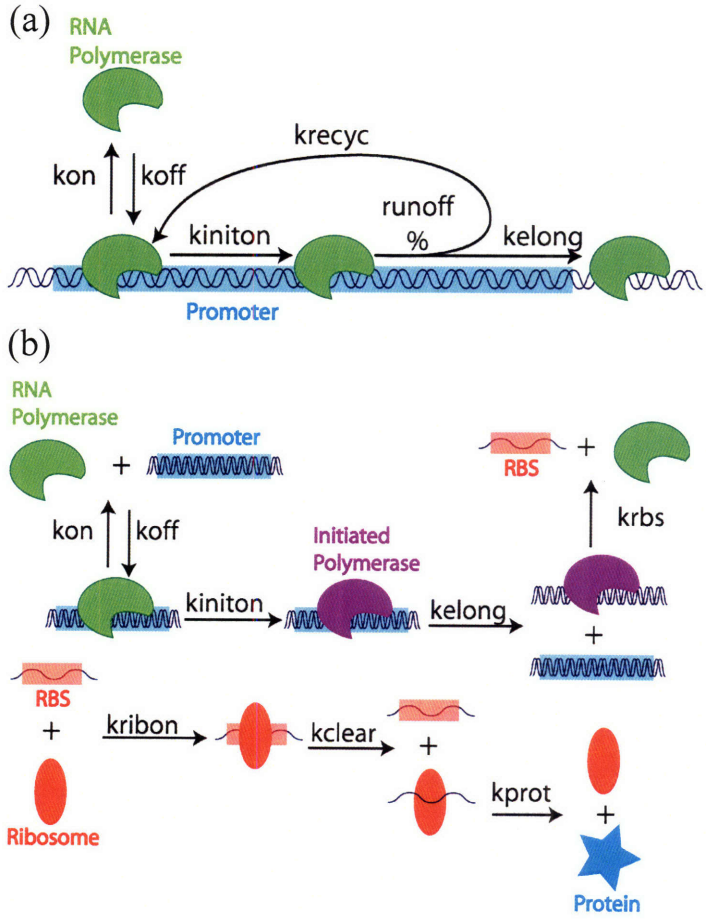


Figure 2-6. Different models representing gene expression. We can represent a simple model for gene expression differently based on the simulator. The Tabasco simulator treats expression at the molecular level. The molecular-level Tabasco-based simulation uses the general schematic used in Figure 2-1, and transcription initiation as shown in (a). The species-level simulation uses the schematic shown in (b). Care was taken to make sure the constants were equivalent in both simulations.

Constant	Tabasco	Species-Level
kon ($M^{-1}s^{-1}$).	4e7	4e7
koff (s^{-1}).	4	4
kiniton (s^{-1}).	1.2	1.2
% runoff	0	N/A
krecyc (s^{-1}).	0	N/A
kelong (s^{-1})	0.23	0.23
kprot (s^{-1})	0.645*	0.645
kribon ($M^{-1}s^{-1}$)	1.15e4	1.15e4
kclear (s^{-1}).	0.14*	0.1308
rib elongation (s^{-1})	20	20
mRNA deg (s^{-1}).	2.5e-3	2.5e-3
protein deg (s^{-1})	7e-4	7e-4

Table 2-1. Constants used in simple gene expression model. The two models tested used the both a species-level SSA and Tabasco to represent gene expression for a simple system shown in Figure 2-6. The Tabasco constants for *kprot* and *kclear* were adjusted to ensure the steady-state levels of mRNA and protein were equivalent in both simulations. The constants do not exactly match because of small variations in the structure of the simulator.

Chapter 2.5.5.3. Polymerase Interactions & Bacteriophage T7

The simulations for the polymerase interactions and bacteriophage T7 used parameters that can be found in the input files in the Appendix [§7.3.2]. The parameters used were based on the derivations described in the next chapter [§3.6]. However, in general, the exact values of the constants are ancillary to this section, which is to show that Tabasco is able to simulate processes such as polymerase interactions and entire genetic systems such as T7.

Chapter 3. Models

Chapter 3.1. Introduction

Biological systems are physical systems that replicate themselves faithfully. The precision and reproducibility of biological behaviors such as development imply reproducible molecular events that govern their processes. However, precise knowledge of an entire biological system with all the numbers, interactions, and dynamics of individual components does not exist. Through genetics, we have information on identity, essentiality, and connectivity of some of the components of biological systems. Biochemistry provides detailed kinetics on individual components of these pathways. Sequencing and modern bioinformatics give us further information on identifying uncharacterized components and the components' evolutionary relationships. However, few successful examples exist for combining this knowledge to construct accurate, biophysically realistic models of entire organisms [Endy & Brent, 2001]. Such models could be used to make model-based predictions to explore questions such as why a particular organism is designed the way it is, and give us better substrate from which to engineer future biological systems.

Bacteriophage T7 is a convenient organism from which to develop such system-level models for several reasons [§1.2]. *First*, T7 is well studied. The genome has been sequenced, annotated, and many important factors involved in RNA processing, DNA replication, and protein expression have been described in detail [Dunn & Studier, 1983; Studier & Dunn, 1983]. *Second*, compared to other pathogens, T7 is relatively independent of host physiology. T7 encodes phage-specific RNA and DNA polymerases, inhibits transcription and translation of most host genes early in infection, and degrades most of the host genome. *Third*, transcription and replication of the T7 genome are mostly independent processes, thus simplifying analysis of gene expression [Zhang & Studier, 2004]. *Fourth*, complete internalization of the T7 genome takes under 10 min at 30°C, or about one third of the latent period [Zavriev & Shemyakin, 1982; Garcia &

Molineux, 1995]. As genes obviously cannot be transcribed before they leave the phage capsid and enter the cell, the slow entry of the T7 genome also provides a direct mechanism for regulating the timing and levels of gene expression. Based on their spatial location and temporal expression patterns, T7 genes are divided into three classes, which also correlate with the gene's function [Studier, 1972]. Class I genes (genes *0.3* to *1.3*) are expressed first and mostly transcribed by the *E. coli* RNA Polymerase. The class II genes (genes *1.4* to *6.3*) followed by the class III genes (genes *6.5* to *19.5*) are expressed next and mostly transcribed by the T7 RNA polymerase.

Previous modeling efforts encoded the aggregate knowledge of T7 biology to make computer simulations of an entire biological organism [Buchholtz & Schneider, 1987; Endy et al., 1997]. These simulations provided encouraging results, but also showed a number of troubling inconsistencies with experimental measurements. Using the model to predict the effects of gene repositioning led to failures in certain predictions of phage growth [Endy et al., 2001]. For example, protein synthesis of most class I and II genes was observed to spike and then fall-off as infection proceeded, whereas the model predicted sustained expression. The model assumed that T7 mRNA was stable based on previous measurements that showed that the total cellular RNA lifetime is greater than 20 minutes during infection [Summers, 1970]. However, individual transcript degradation could be obscured in such bulk mRNA measurements and perhaps is the cause for drop-off in protein synthesis rates.

This chapter describes our work to revisit these original models, specifically focusing on the program of gene expression during T7 infection [Keller et al., *in preparation*]. To do this work, I developed and used a new simulator, Tabasco, to explore in greater detail mechanisms that are thought to govern phage gene expression [§2]. I coupled this analysis with Heather Keller's real-time RT-PCR measurements of the absolute RNA levels over the course of infection for 21 of the 56 known T7 genes. The measurements strongly indicate that T7 mRNA is decaying during infection. I used the model to estimate degradation rates and show that the resultant mRNA profiles correct the previous discrepancies in protein synthesis rates. I use the quantitative mRNA measurements to further constrain different assumptions in the model, and discuss what challenges lie ahead.

Chapter 3.2. Background on T7 Gene Expression

Chapter 3.2.1. General

Bacteriophage T7 is an obligate lytic phage that infects B strains of *E. coli* [Dunn & Studier, 1983; Studier & Dunn, 1983]. The initial genetic studies of T7 found 19 genes to be essential for the viability of the phage, which were numbered 1-19. Further screens and eventually the sequencing of the DNA revealed more genes, which were numbered appropriate to their locations relative to the original 19. In total, the 39,937 base pair (bp) genome encodes 56 genes predicted to specify 60 proteins, of which 45 have been observed by polyacrylamide gel electrophoresis. These genes are separated into three classes called early (class I), middle (class II), and late (class III), depending on their location on the genome. To clarify the naming conventions, the genes are simply written as gene *1* or gene *0.3*. The protein products are labeled gp1 or gp0.3 (for gene product 0.3). Promoters specific to the *E. coli* RNA polymerase (RNAP) are labeled with a capital letter sometimes followed by a number such as A1 or B. Promoters specific to the T7 RNA polymerase (gp1) are labeled with a 'ϕ' in front of an italicized number representing the gene the promoter precedes (e.g., ϕ*10*).

Chapter 3.2.2. Gene Regulation by DNA Entry:

T7 development begins with adsorption of the phage particle to the *E. coli* lipo-polysaccharide, creation of a channel for DNA entry (~1 min), and entry of DNA at a rate of ~75 base pairs per second (bp/s) [Garcia & Molineux, 1995; Molineux, 2001]. Wild-type T7 DNA translocation effectively stops after 850bp has entered the cell, while the remainder of the genome is internalized by transcription, first by RNAP at 40 bp/sec and then by gp1 at ~200-300 bp/sec [Garcia & Molineux, 1995]. Complete internalization of

the T7 genome takes under 10 min at 30°C, or about one third of the entire developmental cycle.

Genes cannot be transcribed before they enter the cell, thus, the slow entry of the T7 genome regulates the time of first expression (e.g., early genes are expressed first, etc.). The organization of gene functions on the genome are mostly grouped by when they are expressed. The early genes, whose functions involve evading host restriction, expression of gene *1*, and blocking *E. coli* RNAP activity, are among the first to enter the cell and thus the first to get expressed. Whereas genes that control particle morphogenesis, DNA packaging, and cell lysis, are all middle or late genes and consequently their expression is prohibited during early infection.

Chapter 3.2.3. Gene Regulation by Promoter Specificities and Strengths:

The amount of gene expression is dependent upon the type, strengths, and locations of the promoters on the genome. Class I genes are expressed early by RNAP using the class I promoters, while class II and then class III promoters are utilized by gp1 later during development. Using one or more of three strong class I promoters (A1, A2, A3), RNAP transcribes about 7 kb containing the first 10 genes of T7, including gp1. Two weaker promoters (B and C) provide no detectable transcripts of T7 DNA *in vivo* but are known to be active in the absence of the A promoters. Transcription by RNAP is thought to stop at the early terminator TE, which is ~ 80% efficient *in vitro*, or at least 90% *in vivo* [Chamberlin, et. al., 1979; Dunn & Studier, 1980; Briat & Chamberlin, 1984]. Gp1 transcribes both class II and III genes. Ten T7 promoters lie within the class II region of the genome, fewer are present in close relatives of T7 but the significance for any given number of class II promoters is unclear. Transcription from all 10 promoters terminate, both *in vitro* and *in vivo*, at ~ 80% efficiency at Tø, the major late transcriptional terminator. Five 23-base conserved class III promoters direct primary expression of all late genes, three of these lie upstream of Tø and thus provide

overlapping transcripts with those from class II promoters. Class III promoters are, however, stronger than their class II counterparts because they are less susceptible to abortive initiation [Ikeda, 1992; Lopez et al., 1997]. Two additional T7 promoters lie close to each genome end; *øOL* is thought to be very weak and does not yield detectable transcripts from T7 DNA *in vivo* but it is known to be active in certain mutant infections [Moffatt & Studier, 1988; García & Molineux, 1995]. *øOR* has a class III consensus sequence but transcribes only a single non-essential gene, and thus its possible role in DNA replication and DNA packaging is likely more important than its transcriptional activity.

Chapter 3.2.4. Gene Regulation by Protein-level Feedback Mechanisms:

T7 gene expression is regulated by feedback controls enacted at the level of protein-protein interactions. First gp0.7, through an unknown mechanism, and then gp2, by direct binding, act in concert to shut down transcription by RNAP and thus expression from host DNA [Nechaev & Severinov, 1999; Hesselbach & Nakada, 1977b; Brunovskis & Summers, 1972]. This feedback mechanism also allows for further differentiation between class I genes and those genes transcribed by gp1 (class II and class III genes). Later in the infection cycle, T7 lysozyme (gp3.5) inhibits the function of gp1 by the formation of a 1:1 complex. At, low gp3.5 concentrations, class III promoters are preferentially expressed over class II promoters [Villemain & Sousa, 1998], whereas at high concentrations of gp3.5, most transcription is repressed and only short abortive transcripts, which are involved in the initiation of T7 DNA replication, are produced [Moffatt & Studier, 1987; Zhang & Studier, 1997, 2004]. Because gp3.5-mediated inhibition of gp1 is required to initiate DNA replication, most phage mRNA is produced from the original infecting copy, and not subsequent copies of the DNA. Thus unlike most phage, T7 DNA replication and late transcription are not coupled (at least at high multiplicity of infection). RNA synthesis, as measured by ^{3}H-uridine incorporation, is

mostly finished by the start of DNA replication, as measured by ^{3}H-thymidine incorporation [Zhang & Studier, 2004]. This aspect of T7 biology is particularly useful because T7 gene expression be studied and modeled fairly independently of DNA replication, capsid assembly, packaging, and lysis.

Chapter 3.2.5. Other Factors:

Little is known about regulation of T7 gene expression at the level of translation other than it is likely to occur. For example, experiments *in vivo* and *in vitro* show that gene *0.3* mRNA is out-competed for translational machinery by late T7 mRNA [Strome & Young, 1978,1980a,b]. Due to the number of overlapping genes, translational coupling may play a role as well. T7 mRNA seems to be stable in *E. coli*, possibly by the modification of the major mRNA degradation enzyme in *E. coli*, RNase E [Summers 1970; Marss & Yanofsky, 1971, Marchand et. al., 2001]. In addition, there are a number of RNase III recognition sites on the genome that may stabilize mRNA transcripts *in vivo* [for review, see Grungerg-Manago, 1999]. Also, RNase III activity is increased upon phosphorylation by gp0.7 [Mayer & Schweiger, 1983].

Chapter 3.3. Results

Chapter 3.3.1. Absolute Measurements of T7 mRNA Transcripts

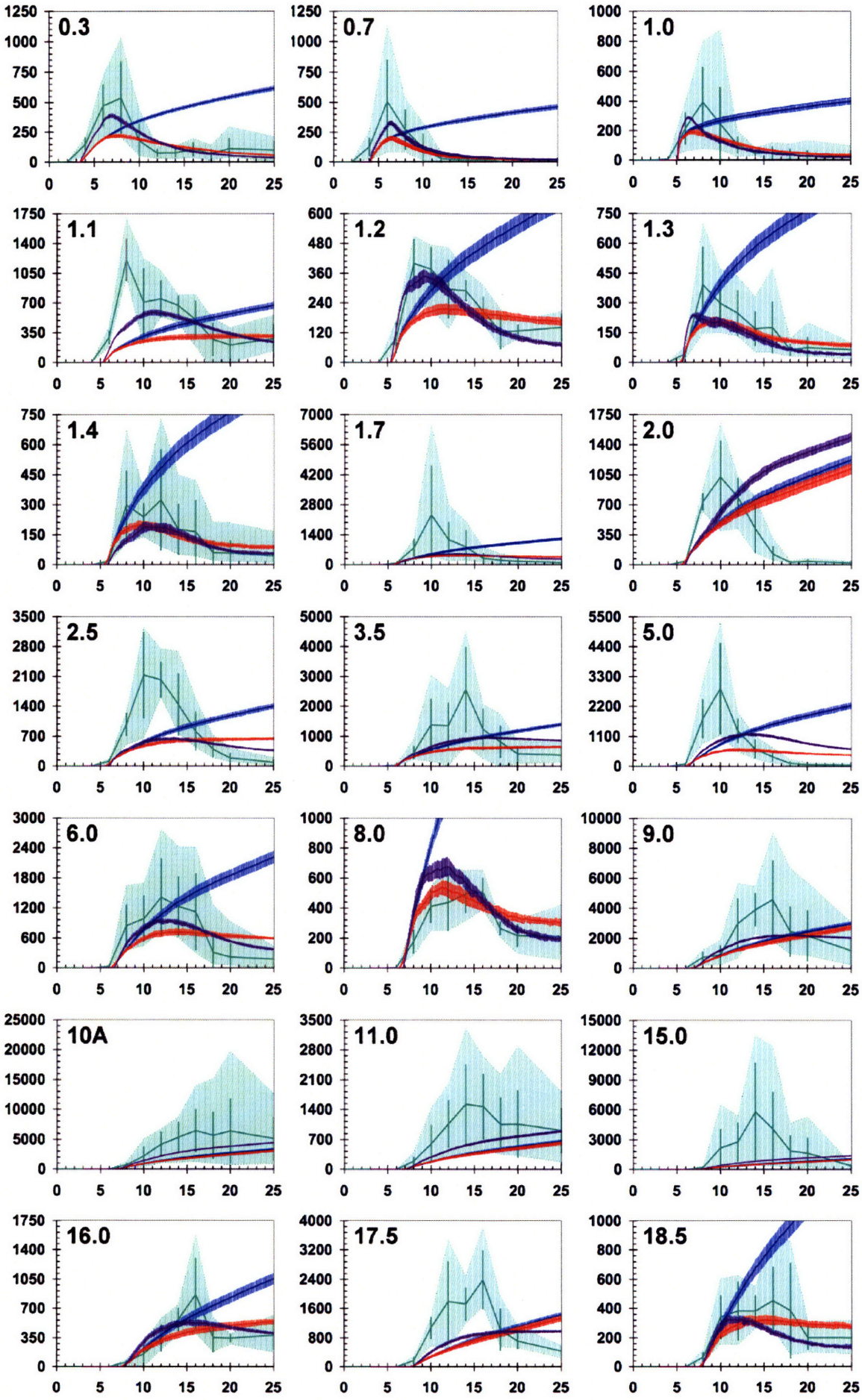
For completeness, I describe Heather Keller's measurements of T7 mRNA during infection [Keller et al., *in preparation*]. Keller used real-time RT-PCR to measure 21 T7 genes over the course of infections. To help constrain the model, this study measured absolute numbers of the average number of mRNA copies per cell. To calculate absolute numbers of mRNA, known amounts of *in vitro* transcribed RNA were used to generate gene-specific standard curves that the experimental samples were compared against. In

addition, exogenous *B. subtilis* mRNA was spiked into the crude RNA samples in order to account for differences in extraction efficiencies between samples.

The real-time RT-PCR measurements agree with previously reported trends in the timing of T7 gene expression. As shown in Figure 3-1, the timing of expression is directly correlated with genome position; class I genes are expressed first and show early peaks, followed by class II and then class III genes. This pattern of expression is in accordance with the prevailing theory regarding T7 genome entry with expression proceeding from left to right across the genome [Zavriev & Shemyakin, 1982; Garcia & Molineux, 1995]. Additionally, the timing of the measurements closely matches previous gene expression studies which have shown that at 30°C: class I RNA synthesis begins 0-4 minutes and ceases 4-8 minutes post-infection; class II transcription begins 4-8 minutes and falls 8-12 minutes post-infection; class III genes are transcribed between 8 minutes post-infection and lysis, with the expression of genes *8*, *9*, and *10* initiating with class II genes, but persisting through late times of infection [McAllister & Wu, 1978].

In addition to the timing of expression, the relative amounts of mRNAs agree well with expectation. Four of the class III genes measured encode key structural components of the T7 phage virion, including the head-tail connector (gene *8*), the scaffolding protein (gene *9*), the capsid protein (gene *10*), and one of the tail proteins (gene *11*). Each T7 virion requires 12, 139, 415, and ~12 copies of these proteins respectively [Endy et al., 1997; Kemp et al., 2005]. Our mRNA measurements show that the peak expression levels of these genes correspond with the levels of protein required during infection, with gene *8* < gene *11* << gene *9* < gene *10* [Figure 3-1]. In addition to quantifying individual transcript levels, the real-time RT-PCR approach allows us to probe other characteristics of T7 gene expression, such as terminator efficiency. T7 genes *11* and *12* are only transcribed when the T7 RNA polymerase reads through the Tø terminator downstream

Figure 3-1 (next page). Real time measurements and simulation results showing absolute, gene-specific mRNA levels per cell during T7 infection. Each plot shows the measured (green) and computed (red;blue;purple) number of T7 mRNA per cell throughout infection. Measurements were made using real-time RT-PCR. The solid lines show the average all of all replicates (2-4 extractions per gene, each extraction measured in triplicate). Shaded regions represent the range of the measurements, with the standard deviation show by error bars. Simulation data represents the Unfit model (blue), initial fit (red), and best fit model (purple; Traffic Jam model with increased promoter strength). The width of the computed curves shows the variation (± standard deviation) from the average of 25 simulations.


0.3
0.7
1.0
1.1
1.2
1.3
1.4
1.7
2.0
2.5
3.5
5.0
6.0
8.0
9.0
10A
11.0
15.0
16.0
17.5
18.5

of gene *10*. The ratio of the gene *11* to gene *10* measurements (between 6 and 25 minutes post-infection) indicates an *in vivo* terminator efficiency for the phage specific terminator Tø. The measured average value of 78% ± 5% is consistent with previous *in vitro* measurements of ~80% [Lyakhov et al., 1997]. Previous *in vivo* measurements on plasmids give a range of termination efficiencies when transcribing T7 DNA (66-98%) depending on the promoter used to initiate transcription [Macdonald et al., 1993, 1994].

Unlike previous reports, many of the T7 genes measured show reduction in absolute transcript levels during middle and late infection, which is visually apparent in Figure 3-1. 19 of 21 genes show meaningful reduction from the peak expression levels (as measured by a Student T-test) [Keller et al., *in preparation*]. The rates of decline are transcript-specific with the class III genes displaying the greatest stability.

Measurement locations within genes were chosen to ensure specificity of the primers; consistent positioning within the gene was not a design criterion. To test the possibility that different regions of a given transcript show non-uniform behavior, Keller designed a series of five primer pairs spaced throughout gene *1*. Each primer pair displayed different absolute levels of expression for gene *1*. For example, the peak expression at 10 minutes for gene *1* showed the most difference between measurements ranging from ~300 to ~1700 copies of RNA per cell [Keller et al., *in preparation*]. However, the shape of the expression curves are consistent throughout infection. Thus, while the absolute levels of mRNA may be affected by measurement location, the observed reduction in relative mRNA levels is still significant.

Chapter 3.3.2. Molecular Model for T7 Gene Expression

I constructed a model for T7 gene expression using Tabasco. Tabasco tracks individual molecules of phage DNA inside the cell and the polymerases transcribing the DNA at single-base resolution [§2]. The initial model was parameterized using empirical results combined with some prevailing assumptions of T7 biology [§3.6]. I did not adjust parameters *a priori* to account for system level data.

I compared mRNA levels of our measurements to output from the simulations based on our initial model [Figure 3-1]. Notably, given the available information prior to the mRNA measurements, the initial model did not encode mechanisms for RNA degradation. The computed mRNA values are, for the most part, overestimates of the measured values and the average R^2 (fraction of variance explained) is 0.2645 [§3.5.3]. However, the timings for the initial rise in measured mRNA levels did match the simulation well [Figure 3], indicating the prevailing model for RNA polymerase mediated genome entry is consistent with the mRNA observations [Zavriev & Shemyakin, 1982; Garcia & Molineux, 1995]. In addition, the timing of transcription initiation and shutoff agree with published results [Figure 3-2; Zhang & Studier, 2004].

Based on observed degradation of measured mRNA, I decided to incorporate mRNA degradation into the model. I initially simulated a range of degradation rates constant for all genes (*data not shown*). These simulations did not produce useful improvements in the comparisons. At low degradation rates the simulations could not account for the fast decline of class I and II mRNA, and at high degradation rates the simulations could not produce high levels of class III mRNA. I next attempted to estimate degradation rates by fitting a range of transcript-specific degradation rates to the measured data (called Initial fit). Currently, an exhaustive search of degradation rates is computationally unfeasible. Thus, I developed a fitting procedure that determines degradation rates of genes from left to right across the genome [§3.5.3]. While not exhaustive, comparisons between measured and fit mRNA levels were visually better and the average R^2 was 88% better (0.4641 versus 0.2645) [Figure 3-1, red; Figure 7-2a; §3.5.3; §7.3.3]. The results for individual genes and estimated half-lives for transcripts are described in Table 3-1. In general, class I genes were the quickest to be degraded, followed by class II genes, and most class III genes showed very little degradation. There are exceptions to this trend, the most apparent of which are genes *1.1*, *8*, and *18.5*. Finally, previous models and simulations, which did not account for mRNA degradation, failed to recapitulate the experimentally observed drop in class I and II protein synthesis rates [§1.2.3; Figure 1-2; Endy & Brent, 2001; Endy et al., 2000]. The simulations fit to the mRNA measurements produced better correspondence to the observed protein synthesis rates [Figure 3-3].

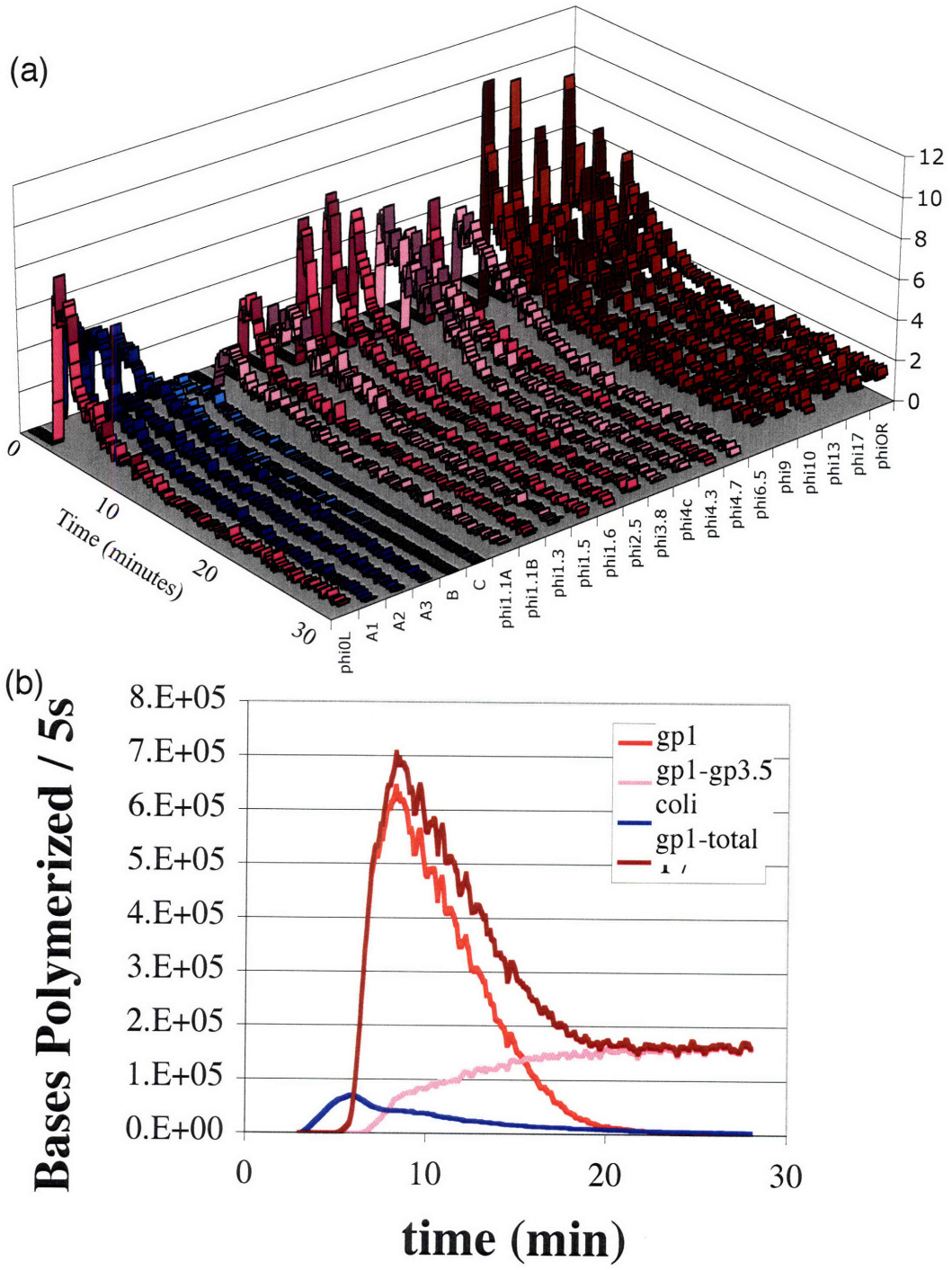


Figure 3-2. Simulated transcriptional activity during T7 infection. Transcription initiations over time (a) from strong (dark blue) and weak (light blue) *E. coli* promoters as well as the three classes of T7 promoters (strong -- dark red; weak processivity -- magenta; weak processivity and binding -- pink) show initial rise at expected timings followed by shutoff later in infection. The attenuation of promoter activity is reflected in the RNA bases polymerized during infection by each of the polymerases (b), and correspond with published results [Zhang & Studier, 2004]. Note the simulations do not account for transcription from the host genome.

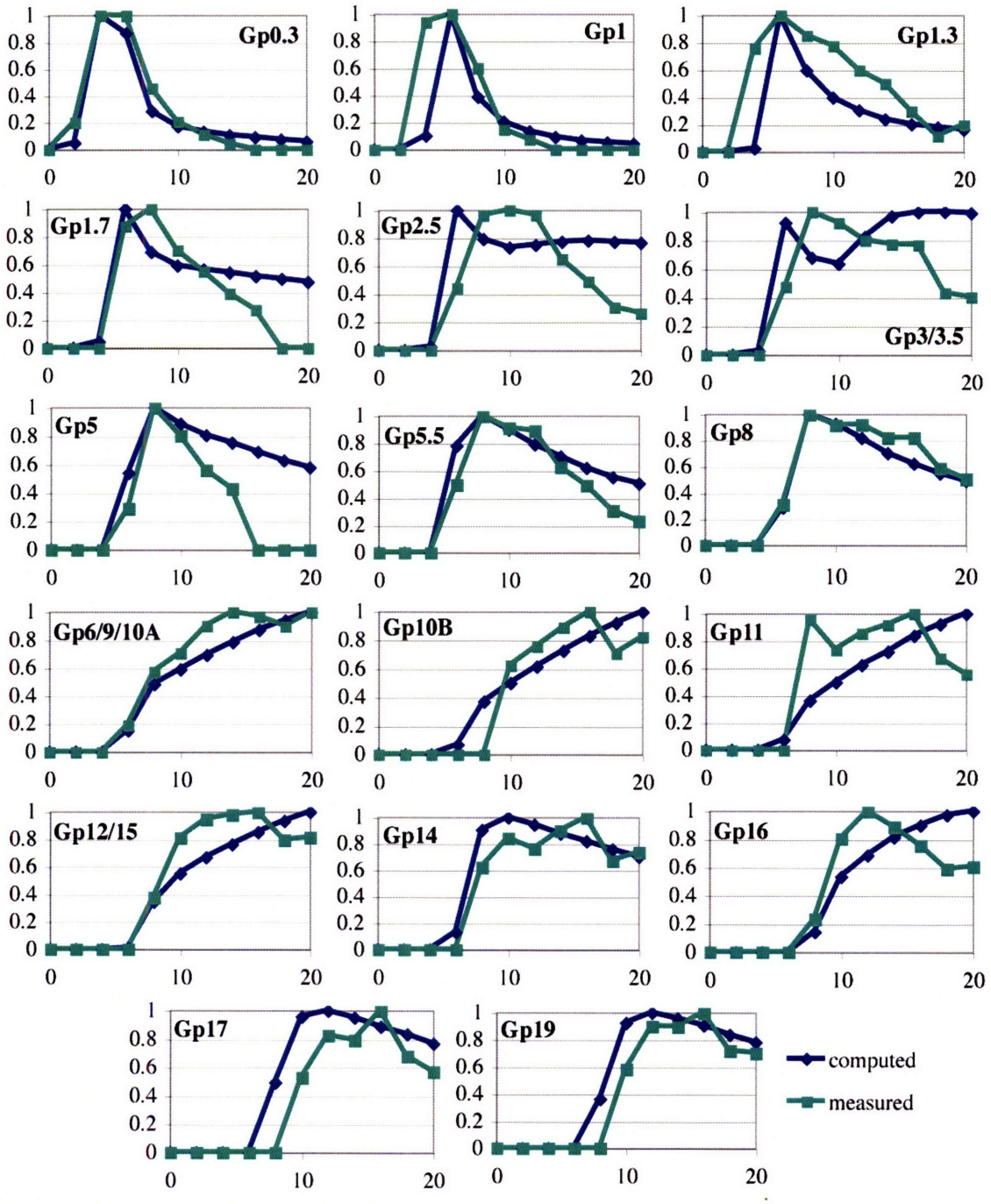


Figure 3-3. Simulated and Measured Protein Synthesis Rates. Simulations (blue) match previous experimental results [Endy & Brent, 2001] showing shutdown of class I and II mRNA much better than previous models (Figure 1-1) when the simulations are fit to mRNA degradation rates. The simulations shown are from the Initial fit.

Chapter 3.3.3. Constraining Models of T7 Gene Expression

I wanted to test the ability of the new RNA measurements to constrain areas of uncertainty in the models. I tested different assumptions used in the model concerning promoter strengths, RNA degradation, and polymerase interactions. Generally, I would alter a particular assumption, and then test the ability of the new simulations to fit the mRNA data shown. I hoped that altering the assumptions and looking at how the comparisons were affected would eliminate some hypotheses.

I observed that simulated RNA polymerase initiation rates indicated that the encoded high abortive initiation percentage was limiting the transcription initiation rate (data not shown). The host and phage promoters on the T7 genome are among the strongest known prokaryotic promoters [Lopez et al., 1994]. I suspected that the measured *in vitro* rates of abortive initiation used in the simulation are higher than the actual *in vivo* rates. I tested the effect of increasing promoter initiation rates of all the promoters [§3.6.5]. I then refit individual degradation rates to the measured mRNA levels [Table 3-1; Figure 7-1a; Figure 7-2b]. Overall, the simulations with increased RNA polymerase initiation rates give an average R^2 that is only 14% better than the originally encoded rates. Most of this change was due to better fits for the class I genes, which showed an average R^2 that was 30% better.

I next tested different RNA degradation mechanisms in the simulations. The Initial fit assumed the degradation machinery competes with ribosomes for the ability to degrade mRNA [Yarchuk et al., 1991, 1992]. Thus, an mRNA with a bound ribosome binding site is immune to degradation [Table 3-1; Figure 7-1b; Figure 7-2c]. I also tested a scenario where mRNA degradation was independent of translation initiation. I found that the simulations assuming competition for RNA showed moderately better fits for class I genes (14%), but the class II and class III mRNA fits showed little change (4% better each).

Finally, I simulated different outcomes resulting from RNA polymerase interactions. Transcribing T7 RNA polymerases are expected to interact with *E. coli* RNA polymerase during infection, because T7 RNA polymerase is measured to have a

five-fold higher synthesis rate [Garcia & Molineux, 1995]. I simulated, fit, and compared four separate hypotheses of polymerase interactions. *First*, upon collision, the downstream transcribing polymerase could terminate transcription, allowing the upstream polymerase to pass (termed DSF for DownStream Falloff) [Table 3-1; Figure 7-1c; Figure 7-2d]. Incomplete mRNA being transcribed from the terminated polymerase are also degraded, and ribosomes translating the nascent mRNA are freed. *Second*, the upstream

half life	nodeg	init	prom	AltDeg	DSF	traffic	USF
0.3	0.11	4	3	4	3	2	2
0.7	0.18	2	2	4	2	1	1
1	0.19	4	4	5	6	1	2
1.1	0.17	15	9	13	15	5	Inf
1.2	0.05	7	6	7	7	2	8
1.3	0.04	3	3	3	4	1	2
1.4	0.06	3	3	3	4	2	2
1.7	0.48	8	6	9	8	3	15
2	0.50	Inf	Inf	Inf	Inf	Inf	Inf
2.5	0.18	14	11	12	15	6	Inf
3.5	0.43	14	14	Inf	Inf	15	Inf
5	0.15	5	5	6	6	4	12
6	0.17	7	6	6	8	4	Inf
8	0.01	3	2	3	4	2	14
9	0.47	Inf	Inf	Inf	Inf	14	Inf
10A	0.54	Inf	Inf	Inf	Inf	Inf	Inf
11	0.44	Inf	Inf	Inf	Inf	Inf	Inf
15	0.48	Inf	Inf	Inf	Inf	Inf	Inf
16	0.23	14	10	13	15	7	Inf
17.5	0.24	Inf	Inf	Inf	Inf	15	Inf
18.5	0.07	4	5	5	5	2	13
class I avg	0.0	3.9	3.5	4.8	4.1	1.4	2.3
class II avg	0.0	7.4	6.8	8.1	9.5	4.5	10.8
class III avg	0.0	12.2	10.0	13.1	15.5	6.2	53.9
class I R^2	0.12	0.44	0.57	0.48	0.43	0.60	0.39
class II R^2	0.28	0.46	0.52	0.50	0.44	0.50	0.36
class III R^2	0.31	0.48	0.51	0.49	0.42	0.53	0.32
total R^2	0.2465	0.4641	0.5295	0.4905	0.4299	0.542	0.3541

Table 3-1. Estimated half-lives for mRNA and R^2 for each gene. T7 genes are listed in the first column. The second column lists the R^2. Columns 3-8 list the estimated half lives for the initial fit (init), increased promoter strengths (prom), the alternative degradation model (AltDeg), the downstream falloff model (DSF), the traffic jam model (traffic), and the upstream falloff model (USF). Each half-life fit is shaded in one of 5 levels of grey corresponding to R^2 in 0.2 increments (darker indicates better fit). *Inf* refers to no degradation rate, or an infinite half life. The bottom of the table lists geometric mean for half-life and arithmetic mean of the R^2 for each of the classes of genes.

polymerase could also terminate transcription (USF), and incomplete mRNA are treated as in the DSF case [Table 3-1; Figure 7-1d; Figure 7-2f]. *Third*, the upstream polymerase could follow at the speed of the downstream polymerase (Traffic) [Table 3-1; Figure 3-1, purple; Figure 7-2e]. *Fourth*, only an upstream T7 RNA polymerase can cause termination of a downstream *E. coli* polymerase (same as increased promoter strength model) [Table 3-1; Figure 7-1a; Figure 7-2b]. All other interactions in the Hybrid simulations follow the Traffic simulation rules. I fit each of the models separately to the measured mRNA data. The USF simulation was the worst at fitting the data with an average R^2 of 0.35, the DSF simulation was 21% better at fitting the data [Table 3-1]. The Hybrid and Traffic simulations both fit the model well, showing 49% and 53% better fits than the USF model, respectively [Table 3-1]. I did not consider a model where polymerases do not interact, i.e., they pass through one another unimpeded. The internal data model of Tabasco cannot represent two polymerase occupying the same space on the DNA.

Chapter 3.4. Discussion

In this study, the mismatch between previous models and experimental data led us to quantitatively measure T7 mRNA as well as to construct more detailed models of gene expression. Heather Keller measured absolute mRNA levels during T7 infection for 21 representative T7 genes. These measurements agreed with many known aspects regarding the timing and levels of T7 gene expression. However, the observed systematic mRNA degradation is in contrast to many previous studies [Summers, 1970; Mars & Yanofsky, 1971; Yamada et al., 1974a; Yamada et al., 1974b; Yamada et al., 1975]. I constructed a best-faith effort to construct a detailed, unfit, and biophysically realistic model of T7 gene expression during infection. Initial comparisons between the model and the measured mRNA levels showed significant discrepancies, primarily due to model assuming mRNA levels were stable. I developed a procedure to fit degradation rates to individual transcripts to fit the mRNA measurements. This procedure produced estimates of the mRNA half-lives, and a much better overall fit to the data. The fit

simulations also corrected previous discrepancies between the simulated and measured class I and II protein synthesis rates.

Measurement of absolute levels of mRNA provided stricter comparisons to our simulations than relative levels could have provided. I used the mRNA measurements to evaluate different hypotheses related to promoter strengths, RNA degradation, and polymerase interactions. For example, when I tested four different possibilities on the nature of expected polymerase interactions, two could not be optimized to fit the measurements well. However, the large error associated with the absolute RNA levels prevented confident discrimination of models concerning promoter strengths and mechanisms for RNA degradation.

The measurements also question previous work indicating that transcription ceases before DNA replication begins [Zhang & Studier, 2004]. The measurements show transcription late in infection of a region upstream of the first rightward facing host promoter A1. Transcription in this early region begins 10-12 minutes post-infection, at least two minutes after transcription begins from all other measured regions [Keller et al., *in preparation*]. During DNA replication, T7 DNA forms concatemers connecting the left end of the genome to the right end [Watson, 1972]. The late rise of mRNA levels from this early region suggests that transcription from the right end of the genome is continuing through the concatemer junction. The presence of concatemer junctions during transcription indicates that DNA replication is initiated before RNA production is complete. DNA replication during the course of transcription would augment the levels of late gene expression predicted by the model. Further exploration of the timing and extent of DNA replication during infection is needed to verify if the observations are meaningful.

We chose to use real-time RT-PCR to measure RNA levels because of the method's proven sensitivity, specificity, and dynamic range [Wong & Medrano, 2005; Bustin, 2000]. However, we found that many aspects of real-time RT-PCR detract from its usefulness in making absolute measurements for constraining a model. *First*, the measurements show that the exponential nature of PCR leads to large error in absolute measurements, making comparison to a model difficult. *Second*, we found that the location of the measured region within a gene affects the observed RNA levels. We are

unable to distinguish between partial and complete transcripts using the standard 50-150bp regions. Therefore, our measurements are likely overestimates of the functional mRNA levels. Longer amplification regions would decrease the detection of partial transcripts, but also lead to problems with RT efficiency, increased PCR optimization, and reduced throughput. *Third*, real-time RT-PCR is expensive and time-consuming for large-scale studies. Ultimately, real-time RT-PCR is still one of the best methods currently available for accurate and sensitive measurement of mRNA, but better techniques are still needed [Wu & Schwartz, 2007].

Although our T7 gene expression model is more complete, there are still mechanisms that need elucidation before we can confidently make model-based predictions of perturbations such as genome rearrangements. For example, the models often underestimate the RNA levels measured in some late genes such as *10* and *15*. Could this be explained by the concurrence of DNA replication and transcription we have hypothesized? In addition, the simulator cannot match the sharp rise and subsequent fall for some class II genes such as *1.7*, *2*, *2.5*. Might this indicate our lack of understanding of degradation mechanisms and/or promoter strengths? Also, we need a better understanding of how the properties individual genetic elements change when placed in different contexts in order to predict the effects of gene rearrangements. For example, how do mRNA degradation rates change when moving a gene from one position to another? Finally, for gene pairs that are expected to be on the same transcript, such as *1.1*/*1.2* and *15*/*16*, the measurements often show that the downstream genes have significantly lower mRNA levels. Are there processivity effects of the RNA polymerase that I am not taking into account? Further understanding of such questions will bring us closer to making accurate model-based predictions.

We can continue the process of increasing our measurement capabilities and study T7 until we can make predictable changes to the genome. I have also started to work on another approach. Rather than making our computational models more realistic approximations of the natural organism, I am constructing new biological organisms that are closer approximations of our models [§4]. Thus, I am constructing organisms that are better optimized to be understood by insulating and standardizing the genetic elements, as well as removing complicating elements.

Chapter 3.5. Methods

Chapter 3.5.1. Model Parameterization

I used mostly empirical data along with some assumptions of T7 biology to constuct a first-pass model of T7 gene expression. Briefly, I derived canonical rates for transcription and translation from previous empirical studies. I classified and parameterized three classes of T7 promoters and two classes of *E. coli* promoters. Polymerase processivity rates, translation rates, and termination efficiencies were taken from literature values as well. Without better information, all translation initiation sites were assumed to have the same strength. Complete details on the parameterization can be found in §3.6.

Chapter 3.5.2. Simulation

Simulations were executed using the Tabasco simulator [§2.2]. All data shown are for average of 25 runs of each simulation. Simulation of a single infection run takes 2-4 hours on a single processor (AMD Athalon). Input files and scripts are provided in the appendix [§7].

Chapter 3.5.3. Fraction of Variance Explained (R^2)

I used R^2 as a metric for comparing our models and measurements. For each gene and timepoint I summed the mean-squared distance (MSD) from the average normalized simulator value to each of the individual normalized measurement values for that timepoint. Both the simulations and measurements were normalized to the highest measurement or simulation value recorded for each particular gene. I summed the MSD for all the gene's timepoints from 0-25 minutes. I also found the minimal MSD achievable by using the average measured value at each time-point instead of the simulated value in the calculation. Then I divided the minimal MSD by the simulation

MSD across all the timepoints to give an R^2 for a particular gene. The R^2 will equal 1 if the simulations produce the average experimental value for each timepoint. Many comparisons use the *average* R^2, which refers to the average R^2 for all genes in a particular simulation. The average R^2 is not equivalent to calculating R^2 for all the timepoints in all the genes all at once.

Chapter 3.5.4. Fitting mRNA Degradation Rates

I optimized mRNA degradation rates to the measured mRNA levels through an iterative process. I first found an average mRNA degradation rate for all class I genes by fitting a range of degradation rates (from 0-15 minute half-lives in 1 minute increments) and finding local maxima for the R^2. I then set the class I average, and tested ranges for the average class II mRNA degradation rates and then class III rates. After the averages were set for all the classes, I tested ranges and fixed degradation rates for each of the individual genes measured from left (gene *0.3*) to right (*gene 18.5*). Upon inspection, the R^2 for almost all the genes and gene classes showed local maxima (or a maxima at one end of the range) at only one location. Each fit was also inspected visually to make sure that the procedure did not produce obviously unrealistic fits. Finally, I found that fitting the later genes did not affect the fits of the earlier genes substantially as evidenced by the lack of large R^2 changes of the fits of upstream genes after fitting downstream genes.

Chapter 3.6. Model Parameterization

Chapter 3.6.1. Constants for Transcription

Chapter 3.6.1.1. RNA polymerase transcription rate and footprint

The rate of *E. coli* RNA polymerase transcription varies on the conditions and templates of the measurements. For example, the transcription rate has been previously

measured *in vitro* to be 55 base pairs per second (bp/s) at 37°C [Bremer & Yuan, 1968], but 43 or 25-28 bp/s at ~30°C [Manor et al., 1969; Rose et al., 1970]. More recently, *in vivo* studies have measured the rate to be 45 bp/s at 30°C and 24-62 bp/s at 37°C [Garcia & Molineux, 1995; Epshtein & Nudler, 2003]. I use the 45 bp/s measurement in our simulations because the mRNA measurements are done at 30°C. For the T7 RNA polymerase transcription rate, I used previous *in vitro* and *in vivo* measurements to obtain a value of 230 bp/s [Golumb & Chamberlin, 1974a; Garcia & Molineux, 1995].

In addition to the transcription rate, I parameterized the footprint of the polymerase in order to account for when they do or do not block other proteins from accessing the DNA. The T7 polymerase elongation complex keeps a transcription bubble size of ~9 bp, and a potential footprint of ~13 bp [Liu & Martin, 2002]. I used the latter value in the size of the footprint during T7 transcription. *E. coli* polymerase is protected from cleavage by free radicals by ~25 bp [Zaychikov et al., 1995; Nudler et al., 1996; Nudler et al., 1998; Korzheva et al., 2000; Greive & von Hippel, 2005]. I used 15 bp as an estimate for the amount of DNA blocked by all the polymerases.

Chapter 3.6.1.2. Promoters Strengths

E. coli promoter parameterization

There are three major *E. coli* promoters, A1, A2, and A3 that are known to direct most *E. coli* polymerase transcription during infection [Dunn and Studier, 1983]. *In vitro* studies also identified several minor promoters named B, C, D (also known as A0), and E [Minkley & Pribnow, 1973; Delius et al., 1973; Stahl & Chamberlin, 1977]. Due to its leftward directionality, I did not include the D promoter in the simulation. In addition, the E promoter was also ignored due to its location close to the right end of the T7 genome. Very little activity *E. coli* polymerase activity remains by the time the E promoter has entered [Hesselbach and Nakada, 1977].

I used stopped-flow kinetic analysis data to parameterize the A1 promoter [Johnson & Chester, 1998]. Abortive initiation assays show that the minor promoters have approximately a ten fold reduction in binding, which is reflected in our

parameterization by a 10-fold increase in *koff* [Dayton et al., 1984]. Figure 2-6 and Table 3-2 describe the exact parameters used in simulations.

T7 promoter parameterization

Traditionally, the T7 promoters are classified into one of three groups: the class II non-consensus promoters, the class III consensus promoters, and the replication promoters [Dunn and Studier, 1983]. The class III promoters, along with one of the replication promoters, *øOR*, all share the same 23 base pair sequence from –17 to +6 relative to the transcription start site at +1. The class II promoters and the other replication promoter, *øOL*, have mutations from this consensus sequence that are described in Table 3-4. I could not find a biophysical justification to differentiate between the replication promoters from the class II and class III promoters, so I treated *øOL* as a class II, and *øOR* as a class III T7 promoter.

Due to a lack of quantitative data to parameterize each promoter separately, I grouped the promoters into classes that could be parameterized. All the consensus promoters have the same parameters. I classified the non-consensus promoters by their deviations from the consensus sequence into either

Name of Constant	Process Description
kon	Rate of RNA polymerase binding to DNA to form an initiation complex ($M^{-1}s^{-1}$).
koff	Initiation complex releasing to form RNA polymerase and free DNA (s^{-1}).
kiniton	Rate of transition from an initiation complex to an elongation complex (creation of first two nucleotides and bubble formation) (s^{-1}).
runoff %	Percent of elongation complexes that continue transcribing past the promoter region instead of recycling to an initiation complex
kelong	Rate of elongation complex clearing promoter and leaving initiation phase (s^{-1}).
krecyc	Rate of recycling by promoter fall off and rebinding to initiation complex (s^{-1}).

Table 3-2. Description of promoter constants used in model. See Figure 2-6 for the corresponding visual depictions of the constants.

Name of constant	T7A2/A3	T7C	wt gp4.7	wt gp2
kon	1.5E7	1.5E7	as before	0
koff	0.28	2.8	as before	N/A
kiniton	0.19	0.19	as before	N/A
runoff %	100	100	70	N/A
kelong	6	6	as before	N/A
krecyc	N/A	N/A	0.19	N/A

Table 3-3. Constants for the *E. coli* RNA polymerase promoter initiation. See Table 3-2 for an explanation of the constants.

weak processivity, or weak binding and processivity. It is known that the region from –17 to –5 mostly contributes to the free energy of polymerase binding, the –3 to –1 region is mostly important in initial double strand melting, and the +1 to +6 region is important in the formation of a stable elongation complex [Bandwar & Patel, 2002]. Differences in the -3 to +6 region affect the kinetic rate of polymerase initiation similarly by reducing the rate of polymerase initiation [Ikeda, 1992]. Thus, differences from consensus in the binding region (-17 to -5) places the promoter in the weak binding category, and differences in the -4 to +6 region lead to weak processivity. The only exception is the ø*OL* promoter, where the –11 G to A difference was previously characterized as having very little effect both *in vivo* and *in vitro* [Ikeda et al., 1992].

After a preliminary analysis of available kinetic data, I created a framework for promoter initiation in Tabasco in order to reflect the mechanisms thought to control T7 RNA polymerase promoter initiation [Figure 3-2]. I then parameterized each class of T7 promoters above from interpretation of available data. For consensus promoter binding I used a *kon* of 1.82E8 $M^{-1}s^{-1}$ and a *koff* of 0.2 seconds [Bandwar et al., 2002; Ujvari and Martin, 1996]. For weaker binding, I used constants of 6.0E7 $M^{-1}s^{-1}$ and 18 s^{-1} for the

Non-consensus Promoter	differences from consensus	groups
øOL	-11,1	weak processivity
ø1.1A	-17,2,4	weak binding&processivity
ø1.1B	3,4,5	weak processivity
ø1.3	-5,5	weak binding&processivity
ø1.5	-2,3,4,5	weak processivity
ø1.6	-2,3,4,5,6	weak processivity
ø2.5	-1,1,4,5	weak processivity
ø3.8	-13,-12,-11,-2	weak binding&processivity
ø4c	-17,-13,2	weak binding&processivity
ø4.3	-2,3,4,5,6	weak processivity
ø4.7	-17,-16,-13,3,4,5,6	weak binding&processivity

Table 3-4. Non-consensus T7 promoters classification. The T7 weak promoters are split into two classes. The first class has differences in the binding region (-17 to -5) and the processivity region (-3 to 6). The second class has differences only in the processivity region (see text).

kon and *koff* respectively [Bandwar et al., 2002]. Finally, I estimated promoter clearance rates based on the work of Ikeda [Ikeda, 1992].

The constants for the initiation rate to form an elongation complex (*kiniton*), the rate to clear the promoter if it is does not fall off (*kelong*), and the rate at which terminated elongation complexes recycle to the promoter complex (*krecyc*) are all kept constant across the different types of promoters. All these constants were derived from the promoter initiation studies of Jia and Patel [Jia & Patel, 1996]. *Kiniton* was measured directly at a rate of 1.82 $M^{-1}s^{-1}$. I inferred from the study that the the rate of addition of bases +2 to +6 (*kelong*) is 30 $s^{-1}bp^{-1}$, plus the remaining 9 bases for the polymerase to clear the promoter at ~230 bp/s, which gave a total of 5.8 s^{-1}. *Krecyc* represents the addition of 4 bases and then a 1 second falloff step, to give a total rate of 0.88 s^{-1}.

Chapter 3.6.1.3. Feedback inhibition on RNA polymerases

Interactions of the *E. coli* RNA polymerase with gp0.7 and gp2.

The phosphorylation of the ß' subunit of the *E. coli* RNA polymerase by gp0.7 is thought to cause a reduction in host transcriptional activity [Zillig et al., 1975]. Paradoxically, phosphorylation and host shutoff functions are distinct and separable by

	T7 Polymerase			T7 Polymerase + Lysozyme		
Name of constant	**strong**	**wbp**	**wp**	**strong**	**wbp**	**wp**
***kon* (/M s)**	1.82E8	6.0E7	1.82E8	1.82E8	6.0E7	1.82E8
***koff* (/s)**	0.2	18	0.2	0.2	18	0.2
***kiniton* (/s)**	3.5	3.5	3.5	0.875	0.875	0.875
***runoff* %**	70	30	30	42	18	18
***kelong* (/s)**	5.8	5.8	5.8	1.45	1.45	1.45
***krecyc* (/s)**	0.88	0.88	0.88	0.22	0.22	0.22

Table 3-5. Constants for T7 polymerase initiation for different promoter classes. The parameterization of T7 promoters is split into 3 classes respresenting the T7 concensus promoters (strong), and two classes of weak promoters; those with differences causing weak processivity (wp) and those with both weak processivity and weak binding (wbp) [Table 3-4].

mutation [Michalewicz & Nicholson, 1992; Simon & Studier, 1973]. For the purposes of this model, I assumed a generalized catalytic model for reducing host polymerase activity by gp0.7. I encoded the measured 70% reduction in activity [Hesselbach & Nakada, 1977a], by assuming gp0.7 modified host polymerase will abort initiation 30% of the time. I assumed the rate at which phosphorylation occurs to be quick, with an *kon* equivalent to the fast association rate measured in the gp1-gp3.5 interaction (see below). More recent analysis, which was not accounted for in this study, shows that gp0.7 phosphorylation increases rho-dependent transcription termination [Severinova & Severinov, 2006].

Gp2, the protein encoded by gene *2*, strongly inhibits *E. coli* RNA polymerase transcription [Hesselbach et al., 1974; Hesselbach & Nakada, 1975]. From interpretation of data from Hesselbach and Nakada [1977b], the K_d of the gene *2*, host holoenzyme interaction is between 2.8E-8 and 5.6E-8 M, which is ~2-3 times as strong that of the gp1-gp3.5 interaction (92 nM, see below). I assumed that this difference is a reduction in *koff*, to 1.1 s^{-1}, because traditionally *koff* is the determinant in the strength of binding interactions. I chose the higher end of strength because of data showing that the disassociation rate of the complex is quite slow [Hesselbach & Nakada, 1977b]. The transcriptional activity of the gene *2*-host RNA polymerase complexes are assumed to be zero.

Interactions of T7 polymerase with T7 lysozyme.

T7 lysozyme encoded by gene *3.5* inhibits transcription by the T7 RNA polymerase by the formation of a 1:1 complex [Moffatt & Studier, 1987; Zhang & Studier, 1997, 2004]. I use the association and dissociation rates for the T7 polymerase and lysozyme of 3.8E7 $M^{-1}s^{-1}$ and 3.5 s^{-1} respectively [Stano & Patel, 2004]. From Kumar & Patel's study, I estimate that T7 lysozyme's effect on transcription are approximately ~60%, thus reducing the runoff percentage for strong and weak promoters to 42% and 18% respectively [Kumar & Patel, 1997]. In addition, attenuation of the rates of initiation and initial elongation are also observed to be 25% of uninhibited polymerase [Kumar & Patel, 1997]. The effect seems to occur for both class II and class III

promoters equally [Ikeda & Bailey, 1992]. A summary of parameter changes upon lysozyme binding is presented in Table 3-5.

Chapter 3.6.1.4. Transcription Termination

There are three known functional terminators on the T7 genome. Starting from the left end of the genome, terminator TE causes termination of RNAP quite efficiently *in vitro* (>95%) with the addition of an unknown protein co-factor in *E. coli* called tau [Briat & Chamberlin, 1984]. *In vivo* termination rates are also increased by modification of the *E. coli* RNA polymerase by gp0.7 [Zillig et al., 1975; Nechaev & Severinov, 2003; Severinova & Severinov, 2006]. However, T7 RNA polymerase efficiently transcribes past TE [Dunn & Studier, 1980]. Currently, I set the termination rate of *E. coli* RNA polymerase at TE to 100% for reasons discussed in the polymerase interaction section (§3.2.1.5).

There are two specific terminators of T7 RNA polymerase. The first is called Tø located between gene *10* and gene *11* and is about 80% efficient *in vitro* [Lyakhov, et al., 1997]. The T7 lysozyme activity was shown to have no effect on the termination at Tø. I used the *in vitro* termination efficiency for the simulations. The other is the CJ pause site located near the left end of the genome [Golomb & Chamberlin, 1974a]. As this terminator is located upstream of all known promoters, its role in gene expression is probably minimal. Thus, the CJ terminator was ignored for the sake of these simulations.

Chapter 3.6.1.5. Inter-Polymerase Interactions

Several lines of circumstantial evidence points to interactions between host and phage polymerase. First, upstream *E. coli* polymerases that collide with downstream polymerase tend to rescue polymerases from small pauses in a cooperative manner [Epshtein & Nudler, 2003]. In addition, expression of a gene from tandem *E. coli* promoters causes interference of RNAP and attenuates gene expression [Adhya &

Gottesman, 1982; Horowitz & Platt, 1982; Nomura et al., 1985]. I assumed for the sake of these simulations that an upstream polymerase, upon encountering a downstream polymerase, will follow behind and be limited by the speed of the downstream polymerase. This can lead to problems with genome entry however, if there is any read-through by the *E. coli* polymerase at TE, which is why I approximated the termination efficiency at 100%.

Chapter 3.6.2. mRNA Degradation

While there is some evidence of early class I T7 RNA being unstable [Yamada et al., 1974a,b, 1975], it is generally thought that T7 mRNA is quite stable during infection. To begin, I initially assumed for the sake of simplicity, that T7 mRNA does not degrade.

Chapter 3.6.3. Constants for Translation

There is little data available on translational strengths of T7 genes. Thus I assumed that all proteins are transcribed with equal efficiency. I used a simple model for translation initiation. I assumed diffusion limited binding of the ribosome to the RNA with a rate of 1E7 $M^{-1}s^{-1}$ [de Smit & van Duin, 2003], followed by a slow initiation step of 0.3 s^{-1} [Kennell & Riezman, 1977]. I assumed the rate of translation to be constant across all mRNA at an average rate of 15 amino acids per second, taken from the analysis of *in vivo* ribosomal elongation rate experiments [Dalbow & Young, 1975]. Estimates of the physical and chemical footprints of the ribosome range between 30 and 40 bases [Steitz, 1969; Hüttenhofer & Noller, 1994; Yusupova et al., 2001]. I used 40 bases to be prudent.

Chapter 3.6.4. Host Parameters

I assumed the host cell to have an average volume of 8E-16 L [Donachie & Robinson, 1987]. I assumed a value of 1800 molecules of active host RNA polymerases in the cell at the time of infection [Bremer & Yuan, 1968]. I also overestimated the number of ribosomes available for T7 infection in two ways. First, I assumed that no host mRNA translation is occurring at the time of infection. Furthermore, I assumed that 10,000 ribosomes are available to the cell, which is on the high end for a cell with doubling time of ~30 minutes [Bremer & Dennis, 1996].

Chapter 3.6.5. Model Refinements

I tested different models for phage gene expression based on their ability to fit the mRNA profiles by fitting the models to the measured mRNA profiles [§3.5.4]. I first increased promoter strengths of the encoded promoters in T7. Both the *E. coli* strong promoters and T7 promoters are amongst the strongest known promoters known *in vivo* in *E. coli* [Lopez et al., 1994]. The initial parameterization of the promoter strengths were based on *in vitro* measurements, and the initiation rates were limited by the initiation timings. I expected that these values were underestimates of the actual *in vivo* rates, and thus I increased the initiation rates of these promoters to test the effects on the overall fit. The initiation rate (*kiniton*) of the strong *E. coli* promoters was increased from 0.19 per second to 0.4 per second. I increased the T7 promoter strengths by increasing the runoff percentage of uninhibited T7 polymerase to 50% and 100% (from 30% and 70%) for the class II and III promoters respectively. The refinements testing alternative degradation model and the polymerase interaction models are described earlier in the text [§3.3.3].

Chapter 4. Refactoring

This chapter is based on a manuscript I co-wrote and published with Leon Chan and Drew Endy [Chan et al., 2005]. The planning, design, construction, and testing of work done on this chapter was done in conjunction with Leon Chan.

Chapter 4.1. Introduction

In nature, the success of an individual organism depends directly on its ability to continue to exist and replicate. Not surprisingly, natural biological systems appear to have evolved, and continue to evolve, to meet these requirements [e.g., Block et al, 1982; Aho et al, 1988]. However, should we also expect that the 'design' of an evolved organism would be further optimized for the purposes of human understanding and interaction? Evidence drawn from fields outside biology suggests that the answer is no.

For example, consider two different approaches to programming computers and electronics: 'genetic programming' and 'structured design.' In genetic programming, evolutionary algorithms are used to evolve computer software or electrical hardware for a particular task [Koza et al, 2003]. The absolute performance of evolved systems often meets, and sometimes exceeds, that produced by human-directed designs [Spector et al, 1999]. However, so-evolved systems are difficult to understand, fix, and modify for new applications [Jacob, 1977]. By contrast, a structured design process produces systems that, in addition to functioning, are designed to be easy to understand and extend [Abelson et al, 1996]. Not surprisingly, an artifact produced via structured design may not be optimal when evaluated only in terms of absolute algorithmic or physical performance. However, a structured design process can bypass two limitations, direct-descent and replication-with-error, which constrain the designs of evolved systems. Thus, we might paradoxically expect that a structured design process will, when practical, produce artifacts whose designs can 'evolve' more quickly.

Here, I, with Leon Chan, converted the genome of a natural biological system, bacteriophage T7, to a more structured design. Our work was initially motivated by past failures in modeling T7 development (below) and by a desire to better understand how the parts that comprise bacteriophage T7 work together to encode a functioning whole [Kirschner, 2005]. The design approach we used was inspired by the practice of 'refactoring,' a process that is typically used to improve the design of legacy computer software [Fowler et al, 1999]. In general terms, the goal of refactoring is to improve the internal structure of an existing system for future use, while simultaneously maintaining external system function.

Bacteriophage T7 (T7) is an obligate lytic phage that infects *Escherichia coli* [Molineux, 1999,2005]. Scientists isolated T7 and other phage to begin to study the physical characteristics and components of biological systems [Demerec & Fano, 1945]. Genetics, and then biochemistry, enabled the discovery and characterization of some of the individual elements that participate in T7 development. Sequencing of the T7 genome revealed additional elements [Dunn & Studier, 1983], not all of which have obvious functions [§1.2.1; §4.4.1].

Synthesis of the knowledge of individual parts and mechanisms produced descriptive, system-level models for T7 development, from genome entry to phage particle formation [Studier & Dunn, 1983]. Two features specific to T7 biology made the construction of system-level models easier. First, compared to other phage, T7 is *relatively* independent of complex host physiology [§1.2]. Second, RNA polymerase pulls most of the T7 genome into the newly infected cell [Zavriev & Shemyakin, 1982; Garcia & Molineux, 1995]. Polymerase mediated genome entry is a relatively slow process that results in the direct physical coupling of gene expression dynamics to gene position.

More recently, others and I have used computational models of T7 infection to begin to explore questions related to the organization of genetic elements on the T7 genome and the timing and control of gene expression across uncertain physical environments [Endy et al., 1997,2000; You & Yin, 2002a,b]. In using these computational models, some predictions did not agree with experiments [Endy & Brent, 2001]. For

example, a mutant phage expected to grow faster than the wild type grew slower [Endy et al., 2000].

Upon inspection, disagreements between model-based prediction and experiment could have arisen for at least three reasons. First, our models could not meaningfully include unknown functions. For example, disruption of an uncharacterized nonessential gene, *1.7*, appeared to impact DNA replication [Endy et al, 2000]. While differences between expectation and observation can suggest follow-on science, a lack of complete component-level understanding debased our system-level analyses. Second, the boundaries of genetic elements on the T7 genome are more complex than our models of the genome. For example, genes *2.8* and *3* are most easily modeled as separable genetic elements even though the actual genes *2.8* and *3* overlap [Figure 4-1A; Figure 7-4]. Element overlap may also encode uncharacterized function(s) having to do with the regulation of protein synthesis or the coupling of selective pressures during evolution. For example, bioinformatic analysis of microbial genomes suggests that gene overlaps are conserved across evolutionary distance [e.g., Johnson & Chisholm, 2004]. Element overlap also prohibits independent element manipulation. For example, on the wild-type genome, we cannot change the gene 3 ribosome binding site without at least changing the codon usage of gene 2.8. Third, a computer model built with separable parts that encode independent functions can be over-manipulated relative to the actual physical system.

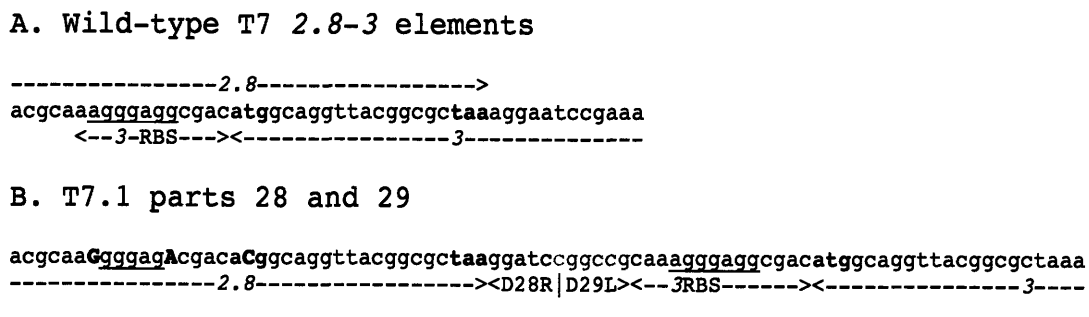


Figure 4-1. Element decompression and part design. (A) The coding regions of genes *2.8* and *3* overlap in the wild-type T7 genome. The ribosome binding site of gene *3* (underlined) is encoded within gene *2.8*. (B) Distinct genetic parts make up the T7.1 genome. The natural ribosome binding site and start codon (green) for gene *3* are disrupted by point mutations (capitals); mutations do not change the amino acid sequence of the 2.8 protein. Parts 28 and 29 are separated by bracketing restriction sites, BamHI (blue) and EagI (orange). Figure S2 lists all changes in the DNA sequence of T7.1 relative to wild-type T7.

For example, while we could simulate the expected behavior of large sets of permuted genomes, we could not easily move a single open reading frame to another arbitrary position on the actual T7 genome [Endy et al, 2000].

Wild-type T7 is a superb organism for discovering the primary components of a natural biological system [Studier, 1972]. However, is the original T7 isolate also best suited for understanding how all parts of the phage are organized to encode a functioning whole? Given our experiences, we decided to attempt to engineer a surrogate genome, which we designated T7.1.

Chapter 4.2.Results

Chapter 4.2.1. Design Goals

Six goals drove our design of the T7.1 genome. First, we wanted to define a set of components that function during T7 development and, for each element, choose an exact DNA sequence that we could use to encode element function. Second, we wanted the DNA sequence encoding the function of any one element to not overlap with the DNA sequence encoding any other element. Third, we wanted the DNA sequence of each element to encode only the function assigned to that element and not any other functions. Fourth, we wanted to enable the precise and independent manipulation of each element. Fifth, we needed to be able to construct the T7.1 genome. Sixth, we needed the T7.1 genome to encode viable bacteriophage; at the start of this work, we were uncertain how many simultaneous changes the wild-type genome could tolerate.

Chapter 4.2.2. Design Process

Briefly, we began design of the T7.1 genome by re-annotating the genome of wild-type T7. The wild-type T7 genome is a 39,937 base pair linear double-stranded DNA molecule [Dunn & Studier, 1983]. We annotated the genome by specifying the

boundaries of the following functional genetic elements: 57 open reading frames with 57 putative ribosome binding sites encoding 60 proteins, and 51 regulatory elements controlling phage gene expression, DNA replication, and genome packaging. Our own re-annotation can be found online at http://openwetware.org/wiki/T7.1/Reannotation.

To specify the architecture of T7.1, we organized the functional genetic elements into 73 'parts.' Each part contains one or more elements. While the DNA sequence of elements within parts may overlap, there is no overlap across part boundaries [Figure 4-1B]. Next, we organized contiguous parts into 'sections' with section boundaries defined by restriction endonuclease sites found only once in the sequence of the wild-type genome. Six sections, *alpha* through *zêta*, make up the T7.1 genome [Figure 4-2A; Figure 7-4]. Sections were used to compartmentalize changes across the genome. In addition, sections can be built, tested, and manipulated independently.

To specify the DNA sequence of T7.1, we eliminated sequence overlap across part boundaries. Overlaps were eliminated by exact duplication of the wild-type DNA sequence; subsequent sequence editing produced a single instance of any duplicated element [Figure 4-1B; Figure 7-4]. All sequence edits within open reading frames were silent and maintained the wild-type tRNA specification or, when necessary, specified a higher abundance tRNA [Ikemura, 1981]. We also added bracketing restriction endonuclease sites to insulate and enable the independent manipulation of each part [Figure 4-2C, E; Figure 7-3]. Bracketing sites are not used elsewhere in the sequence of any one section but are reused across sections. The DNA sequence of T7.1 changes or adds 1,424 base pairs to the wild-type genome [Figure 7-4].

Chapter 4.2.3. Construction & Testing

The sections that comprise the T7.1 genome can be built and tested independently. We constructed the first two sections, *alpha* and *beta* [§4.4.3]. *Alpha* and *beta* contain the first 32 of 73 parts of the T7.1 genome, replacing the left 11,515 base pairs of the wild-type genome with 12,179 base pairs of redesigned DNA, and encoding the entire T7 early region, the primary origins of DNA replication, most of the T7 middle genes, and the

control architecture that regulates T7 gene expression. *Alpha* and *beta* also contain the highest density of elements across the genome. We combined *alpha* and *beta* with the remainder of the wild-type (WT) genome to produce three chimeric phage: *alpha*-WT, WT-*beta*-WT, and *alpha-beta*-WT.

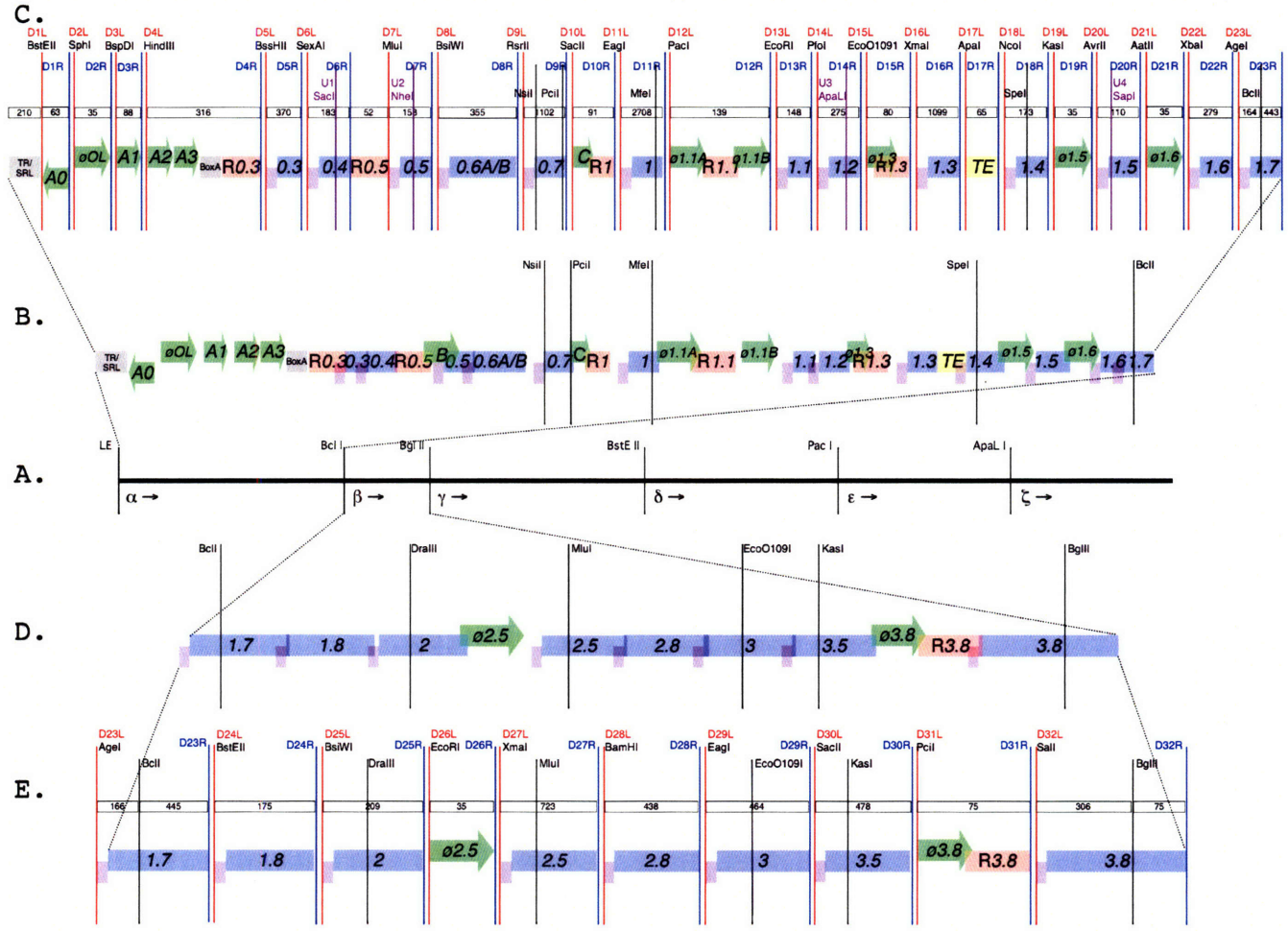


Figure 4-2. Genome design. (A) We split the wild-type T7 genome into six sections, alpha through zêta, using five restriction sites unique across the natural sequence. (B) Wild-type section alpha genetic elements: protein coding regions (blue), ribosome binding sites (purple), promoters (green), RNase III recognition sites (pink), a transcription terminator (yellow), and others (gray). Elements are labeled by convention [7]. Images are not to scale, but overlapping boundaries indicate elements with shared sequence. The five useful natural restriction sites across section alpha are shown (black lines). (C) T7.1 section alpha parts. Parts are given integer numbers, 1 through 73, starting at the left end of the genome. Unique restriction site pairs bracket each part (red/blue lines, labeled D[part #]L/R). Added unique restriction sites (purple lines, U[part #]) and part length (# base pairs, open boxes) are shown. We do not know if sequence changes in and around parts 6 and 7 destroy the minor E. coli promoter, B. (D) Wild-type section beta genetic elements. (E) T7.1 section beta parts. Figure S2 depicts the six sections, alpha through zêta, which make up the T7.1 genome.

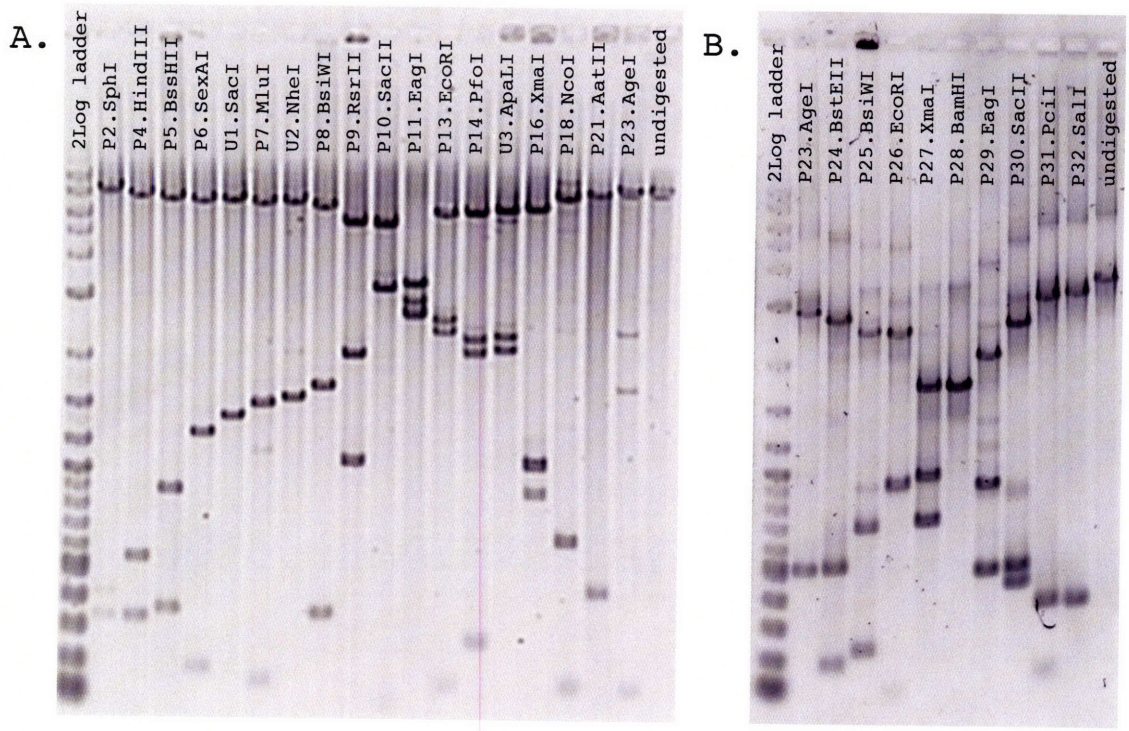


Figure 4-3. Cutting parts from T7.1 (A) Restriction enzymes specific to the sites that bracket parts (P#.Enzyme) and added unique restriction sites (U#.Enzyme) were used to cut section *alpha* (*24*). A subset of the digests is shown. As built, part 1 cannot be removed. (B) Restriction digests cutting out all parts in section *beta*. As built, part 28 cannot be removed.

We tested and recovered viable chimeric phage by transfection and plating. All three chimeric phage are viable. We isolated DNA and performed restriction digests across *alpha* and *beta* to confirm that individual parts could be independently manipulated. 30 of 32 parts in sections *alpha* and *beta* can be cut out as designed [Figure 4-3]. We also sequenced *alpha* and *beta*. Sequencing revealed differences between the design of T7.1 and the actual 'as-built' sections. Relevant sequence differences in section *alpha* include a single base deletion in gene *0.4* and in the *E. coli* terminator TE. Differences in section *beta* include a single amino acid substitution in both genes *1.8* and *2*, a single base deletion in gene *2.5*, and an 82-base truncation in gene *2.8*. All differences were due to errors or limitations in construction [Table 7-1; Table 7-2].

Finally, we characterized some growth properties of the chimeric phage by liquid culture lysis and plating. Phage-induced lysis of log-phase 30°C liquid cultures indicated a 20, -1.4, and 22% increase in the half-lysis times of the *alpha*, *beta*, and *alpha-beta* chimeras, respectively, relative to the wild type [Figure 4-4A]. Plaques were

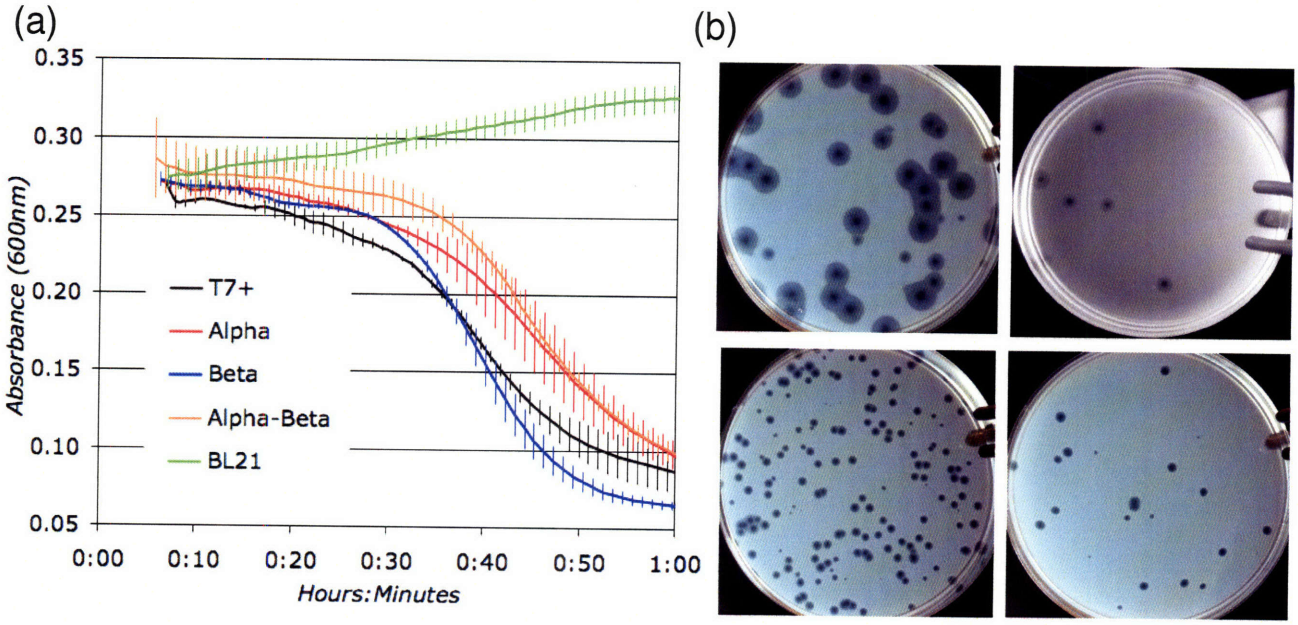


Figure 4-4. Characterization of T7.1 (a) Lysis of log-phase liquid cultures of E. coli BL21 (30°C) by wild-type T7 (black), alpha-WT chimera (red), WT-beta-WT chimera (blue), alpha-beta-WT chimera (orange); absorbance of 0.275 is ~2E8 cells/ml. Vertical bars show standard deviation at each time point (based on four replicates) [Materials & Methods]. (b) T7 plaques on E. coli BL21 (24 hrs, 37°C, 10cm Petri dish). Clockwise from top left: wild-type (WT) T7, alpha-WT chimera, WT-beta-WT chimera, alpha-beta-WT chimera [Materials & Methods].

indistinguishable early during plaque growth and at 30°C (not shown). At 37°C the chimeric phage plaques appeared to stop growing as the bacterial lawns developed; after 24h at 37°C, plaque sizes relative to the wild type were smaller for each of the chimeric phage, with the *alpha-beta* chimera being smallest [Figure 4-4B].

Chapter 4.3. Discussion

A system that is partially understood can continue to be studied in hope of exact characterization [Davis, 1946]. Or, if enough is known about the system, a surrogate can be specified to help study or extend the original. Here, we decided to redesign the genome of a natural biological system, bacteriophage T7, in order to specify an engineered biological system that is easier to study and manipulate. The new genome, T7.1, is based on our incomplete understanding of the information encoded in the wild-type genome and our desire to insulate and independently manipulate known primary

genetic elements. We constructed the first two sections of T7.1 and observed that the resulting chimeric phage are viable.

Phage viability demonstrates the following for sections *alpha* and *beta*. First, our parts as chosen can be separated by exogenous DNA sequence. Second, any functions encoded by part overlap are non-essential. Third, our current understanding of T7 is *not insufficient* to specify a viable bacteriophage. Viability does not demonstrate sufficiency because (i) if the chimeric phage had not been viable then our current understanding would have been demonstrably *insufficient*, and (ii) while T7.1 is based on our current understanding, we do *not* have an exact understanding of all functions encoded in the T7.1 genome (e.g., genes of unknown function). Finally, viability, combined with the observed similarities in lysis times, suggest that T7.1 preserves polymerase-mediated genome entry and remains *relatively* independent of host cell physiology.

The T7.1 genome is easier to model and study. For example, by removing genetic element overlap, the T7.1 genome better matches the understanding of T7 biology encoded in our models, relative to the wild-type phage. However, more work is needed to demonstrate that the dynamic behavior of the system encoded by the T7.1 genome is easier to predict. Such work will benefit from the fact that the parts of T7.1 can be independently manipulated.

We constructed sections *alpha* and *beta* manually. Concurrent advances in de novo DNA synthesis technology have recently enabled the rapid automatic synthesis of DNA fragments the size of the T7.1 genome sections [Stemmer et al, 1995; Yount et al, 2000; Smith et al, 2003; Kodumal et al, 2003; Tian et al, 2004]. As genome synthesis and engineering technologies continue to improve [Carlson, 2003], the use of bracketing restriction sites for manipulating each part should become less important, but the physical separation of parts by the elimination of sequence overlap will remain useful. In general, we expect that the ability to 'write' long fragments of synthetic DNA will directly accelerate the engineering of biology, and impact the science of biology at least as much as large-scale automated DNA sequencing technology.

The viability of T7.1 presents a choice. T7.1 is a 'physical model' that can be used to continue to study the wild-type phage. Observed differences in the behavior of T7.1 relative to wild-type highlight relevant gaps in our understanding of the natural

system. Or, as T7.1 is simpler and easier to manipulate and appears to retain features that make wild-type T7 an attractive system to study, we can define T7.1 as our new model system; successor phage based on T7.1 can be constructed in order to answer questions of genome organization, regulation, and evolution. More generally, other natural biological systems could be redesigned and built anew in support of scientific discovery and human intention.

Chapter 4.4.Methods

Chapter 4.4.1. Re-Annotation of the Wild-Type T7 Genome

Chapter 4.4.1.1. Summary:

We used past experiments and observations to define specific boundaries of functional genetic elements on the bacteriophage T7 genome. We followed the standard naming conventions developed by Studier and Dunn [Dunn & Studier, 1983; Studier & Dunn, 1983]. The discovery of coding domains in T7 is discussed earlier [§1.2.1].

Chapter 4.4.1.2. Control Elements

4.4.1.2.A Ribosome Binding Sites

The definition of a ribosome binding site (RBS) that we used here is a contiguous stretch of DNA that, when transcribed, produces a region of RNA that interacts with the ribosome and allows for the initiation of protein synthesis. The T7 ribosome binding sites were first postulated by analysis of the sequence data upstream of protein coding domain start codons; DNA sequence complementary to the *E. coli* 16S rRNA suggested a functioning RBS. Direct observation of proteins during T7 infection provides additional support for the function of a subset of RBSs [Studier & Maizel, 1969].

4.4.1.2.B RNA Polymerase Promoters

The definition of a promoter that we used here is a contiguous stretch of DNA that interacts with an RNA polymerase molecule and allows for the initiation of mRNA synthesis. At least 22 RNA polymerase promoters help to coordinate transcription dynamics during T7 infection. The discovery and mapping of the RNA polymerase promoters is given earlier [§1.2.1]. The T7 RNA polymerase promoter was determined by sequencing the 23 base pair region common to the late T7 promoters [Boothroyd & Hayward, 1979]. Here, we used a 35 base pair region to define T7 promoters; our broader definition of T7 promoter elements hoped to include conserved regions beyond the initial 23 base pairs [Dunn & Studier, 1983]. The *E. coli* RNA polymerase promoter is less well defined. Here, we used regions of at least 60 base pairs, ranging from the –50 to +10 positions, to define the major and minor *E. coli* promoters (A0, A1, A2, A3, B, C, and E). Also, a *boxA* recognition site located between A3 and gene *0.3* is thought to be involved with anti-termination of polymerases that initiate from the three strong early promoters, A1, A2, and A3 [Olson et al, 1982]. Finally, The cloning of random sections of the T7 genome into a plasmid that selected for transcription activity from the cloned fragment identified other possible promoters [Studier & Rosenberg, 1981]. Sequence analysis in regions containing these sections identified regions of homology to other known promoters [Dunn & Studier, 1983]. Any contribution of these additional promoters to wild-type T7 infection is not now defined. While we annotated these additional promoters, we did not incorporate them as functional genetic elements of T7.1.

4.4.1.2.C RNA Polymerase Terminators

The definition of a terminator that we used here is a contiguous stretch of DNA that, during transcription, produces a region of mRNA that stops the process of transcription (at some efficiency). The first T7 transcription termination site was identified by mapping the endpoints of mRNA starting from *E. coli* promoters [Studier, 1972]. Later it was shown that termination occurred at the same place *in vivo* and *in vitro* [Dunn & Studier, 1973]. The termination site was later mapped precisely, sequenced, and subsequently named 'TE' [Studier et al, 1979; Dunn & Studier, 1980]. A second terminator specific to T7 RNA polymerase was suggested by *in vitro* transcription studies

on digested T7 DNA [Golomb & Chamberlin, 1974; Niles & Condit, 1975]. The terminator, named 'Tø,' was shown to function *in situ* [Dunn & Studier, 1980] and on plasmids [McAllister et al, 1981]. Both TE and Tø have stem loop structures that are thought to set termination efficiency [Dunn & Studier, 1973]. The stem loop and flanking sequence, which includes a poly-uridine tract, were taken together to define the element we used here. While other terminators have been postulated, their precise location and function, if any, during wild-type infection are tenuous [Dunn & Studier, 1983], and thus we did not include them in our annotation.

4.4.1.2.D RNaseIII Recognition Sites

The definition of an RNaseIII recognition site that we used here is a contiguous stretch of DNA that, when transcribed, produces a region of mRNA that is recognized and cleaved (at some efficiency) by RNaseIII. Sites for specific cleavage of T7 RNA by RNaseIII were first shown in vitro and then correlated to *in vivo* data [Dunn & Studier, 1973]. In time, ten RNaseIII sites were mapped and their sites of cleavage identified [Dunn & Studier, 1983]. The sites are thought to stabilize the 3' end of T7 transcripts by providing a stem loop that prevents the binding of scanning single stranded RNA degradation enzymes. A downstream gene often immediately follows an RNaseIII site. Thus, we kept the RNaseIII recognition site elements as short as possible – with a minimum boundary set by the probable stem loop structures [Dunn & Studier, 1983].

4.4.1.2.E DNA Replication Origins

The definition of a DNA replication origin that we used here is a stretch of DNA that is used to initiate the copying of phage DNA during T7 infection. The primary replication origin was mapped to the dual promoter region downstream of *ø1.1A* and *ø1.1B* by analysis of replication bubbles in electron micrographs [Dressler et al, 1972; Wolfson et al, 1972] and subsequently sequenced [Saito et al, 1980]. The secondary origin at øOL was identified using mutants that lacked the primary origin [Studier & Rosenberg, 1981; Tamanoi et al, 1980]. Finally, plasmids containing cloned fragments of T7 DNA were used to screen for regions that act as replication origins during T7 infection; these experiments revealed that øOR and *ø13* have origin activity [Dunn &

Studier, 1983]. While the precise boundaries of the replication origins are unknown, each appears to be linked to a functioning RNA polymerase promoter [Zhang & Studier, 2004]. Here, we only annotate and define an element for the primary origin. While we do not include other replication origins as elements, we do preserve the RNA polymerase promoters that are associated with these secondary origins as elements, and thus possibly the secondary origins as well.

4.4.1.2.F Terminal Repeats & Short-Repeats

The definition of a terminal repeat that we used here is a contiguous stretch of DNA present at both ends of the T7 genome, and a short repeat is a series of direct repeats of DNA near the end of the genome. Both the left and right ends of the T7 genome contain exact 160 base-pair direct repeats [Ritchie et al, 1967]. Also, adjacent to the direct repeats on both ends of the genome are regions of DNA that contain 12 regularly arranged and highly conserved seven base pair sequences termed the short-repeats left, SRL, and right, SRR [Dunn & Studier, 1981]. The terminal repeats and SRL/R are thought to be involved in concatemer formation, DNA packaging, and particle maturation [Kelly & Thomas, 1969]. However, the mechanisms by which the direct repeats and the SRL/R act are unclear. Thus, we treated each end's direct repeat and SRL/R as a monolithic element (the design of T7.1 does not make any changes to the DNA sequence of these elements).

Chapter 4.4.2. Design of T7.1 Genome

Chapter 4.4.2.1. Design Goals & Overview

Three goals drove our design of T7.1. First, we wanted to insulate and enable independent manipulation of all identified genetic elements [§4.2.2]. Second, we wanted the T7.1 genome to encode a viable bacteriophage. Third, we wanted the behavior of T7.1 to be as close to wild-type T7 as possible. The second and third goals acted as constrains on the changes that we attempted in pursuit of the first.

The design of T7.1 genome uses six *sections*, *alpha* through *zêta*. Each section is made up of *parts* that contain one or more *functional genetic elements* [Figure 7-3]. In our design, the modification of parts on the full T7.1 genome is a two-stage process. First, parts are manipulated within a section. Second, sections are combined to assemble a full genome. The design of sections *beta* through *zêta* were improved based on our experience building section *alpha*.

Chapter 4.4.2.2. Definitions

#-Cutter: A restriction enzyme that cuts a particular DNA sequence # times
Functional Genetic Element: A promoter, protein coding domain, ribosome binding site, etc., defined in the re-annotation of the T7 genome [§4.2.2].
Part: A piece of DNA that encodes one or more functional genetic elements and is bracketed by a pair of identical restriction sites.
Construct: Any amalgamation of functional genetic elements or parts.
Section: A segment of the T7.1 genome who boundaries are 1-cutters on the wild-type T7 genome.

Chapter 4.4.2.1. Sections

We used sections to limit the number of simultaneous changes to the wild-type T7 sequence and to make the construction process more manageable. Two practical considerations drove our choice of section boundaries. First and foremost, the boundaries of the sections had to be compatible with the sparse distribution of 1-cutter sites across the wild-type genome. [The use of 1-cutter sites for section boundaries allows refactored sections to be easily combined with other sections or with wild-type DNA.] Second, the number of parts per section was limited by the number of "useful" 0-cutters across the DNA sequence of each wild-type section. Useful 0-cutters are specific, free or smaller recognition sites, *dam/dcm* insensitive, and leave sticky-end overhangs.

Chapter 4.4.2.2. From Functional Genetic Elements to Parts

Parts are made up of one or more functional genetic elements. Parts containing more than one element were sometimes used to maintain the natural proximity of elements known or likely to by physically or functionally coupled. For example, we grouped most ribosome binding sites and downstream protein coding domains into two-element parts. Also, some functional genetic elements overlap so severely as to prevent efficient separation (e.g., the genes *4A*, *4B*, *4.1*, and *4.2)*. Finally, some functional genetic elements were very short (<150bp) such that variants containing deletions or separations of the individual elements could be easily constructed (e.g., the *E. coli* promoter C and RNase III site R*1*). In total, we combined the elements that make up T7.1 into 73 parts. We numbered parts, one to seventy-three, starting from the genetic left end.

The arrangements of parts on the wild-type T7 DNA sequence sometimes resulted in the overlapping of the DNA sequence specifying parts. To remove part-part overlap, we duplicated the DNA sequence of the overlap, providing both parts with an independent copy of the previously overlapping sequence. If, as a result of sequence duplication, either of the parts encoded a function specific to an element in the other part, we mutated the sequence to eliminate the duplicate function. All mutations to protein coding domains were silent and result in either no change in the tRNA or, when necessary, specify a higher abundance tRNA [Ikemura, 1981]. Parts separation is detailed in Figure 7-4.

We surrounded each part with a restriction site pair that is not contained elsewhere in that section. Typically, we added bracketing restriction sites to the DNA sequence of each part but, when appropriate, we integrated the sites into the natural DNA sequence. Also, to help reduce the length of T7.1 and were possible, we chose adjacent restriction sites to have overlapping sequence with one another.

One of the most significant differences between the design of section *alpha* and the other sections was in our choice of bracketing restriction sites. In section *alpha*, we picked restriction enzymes that did not cut within section *alpha* only. However, as the construction of *alpha* proceeded, and cloning directly into the phage became useful, it

was clearly advantageous to use restriction enzymes that did not cut within the entire genome where possible.

Chapter 4.4.2.3. Design Features

4.4.2.3.A Deletion and Insertion

The design of the T7.1 genome allows for the simple deletions of parts. Generally, the section containing the part is isolated and digested by the bracketing restriction enzyme. The fragments can then be ligated to reform the section minus the deleted part, and then joined to the rest of the genome.

Insertion of a new part can be more involved. Most simply, if there is a pre-existing restriction site due to a deletion operation, then a new part can be inserted in its place. If no such site exists, another method involves using two restriction enzymes, NgoMIV and BspEI, that are 0-cutters across both the wild-type T7 and all refactored sections. NgoMIV and BspEI have different recognition sequences but produce the same overhang upon digestion. This allows for ligation of a product into these sites, while simultaneously preventing the restriction sites from being reformed. Thus, a part adjacent to the desired insertion site must be replaced with the same part that has an NgoMIV site appended to it. Then the part to be inserted is amplified with bracketing BspEI sites and inserted into the NgoMIV site. Since neither restriction site is reformed upon insertion, this method can be reused to serially insert parts throughout the genome.

4.4.2.3.B Unstuffing Hooks

Since we didn't know how a phage made of separated parts would behave (e.g., would it form plaques?), we thought that it would be prudent to be able to easily revert to the wild-type T7 sequence for purposes of comparison and debugging. Thus, we used silent mutations to add additional 1-cutter restriction sites to section *alpha*. These new restriction sites, labeled U1-4, can be used to replace refactored regions with wild-type sequence. In sections *beta* through *zêta*, such extra sites were superfluous because we used 0-cutters to bracket parts.

4.4.2.3.C Scaffolds

We used scaffolds to build sections *alpha* and *beta*. A scaffold is essentially the sequence that remains when all parts are removed from the section. As such, the scaffold contains all the restriction sites required to assemble the parts to form the section. In addition, if a fully refactored phage was not viable, we could use the scaffold to incrementally revert the sequence back to wild type in an attempt to restore function.

Chapter 4.4.2.4. T7.1 Annotation and Sequence

GenBank files for the annotation of T7.1 and the as-built section *alpha* and *beta* are available upon request and are being deposited with NCBI. These three files plus a GenBank file of our re-annotation of wild-type T7 are now available online: http://web.mit.edu/endy/www/ncbi/

Chapter 4.4.3. Construction

Chapter 4.4.3.1. Alpha

The design of the scaffold for section *alpha* included all functional genetic elements from the left end of T7 through R*0.3*, R*0.5*, parts 17,19, 21, plus the restriction sites required to add all remaining parts (1334 bp total). The section *alpha* scaffold does not contain any known protein coding domains. We sent the scaffold sequence to Blue Heron Biotechnology for synthesis (http://www.blueheronbio.com/). The scaffold could not be assembled onto the standard cloning plasmids then in use by Blue Heron and us. Blue Heron agreed to instead ship the section *alpha* scaffold as four fragments with point mutations in each fragment. The point mutations were:

Fragment 1: Single base changes at 89(G-T), 168(A-T), 169(C-A), 245(G-A) and 249(C-A) as well as single base deletions at 138 and 159

Fragment 2: A single base deletion in the -35 box of the *A1* promoter

Fragment 3: A four base deletion between the -35 and -10 boxes of the *A3* promoter

Fragment 4: A single base deletion in the loop of TE

We decided to discard Fragment 1 but to correct and make use of Fragments 2, 3 and 4. We built a new vector, pREB, to facilitate the assembly of section *alpha*. pREB (for rebuild) started as a chimera of the inducible copy control system of pSCANS-5 and the insulated multi-cloning site (MCS) of pSB2K3-1 [§4.3.4]. We completed pREB by adding a smaller MCS containing PstI, BstBI and BclI restriction endonuclease sites and by removing nineteen other restriction sites from the plasmid backbone.

To build section *alpha* we first cloned parts 5, 6, 7, 8, 12, 13, 14, 15, 16, 18, 20, 22, and 24 into pSB104. We cloned part 11 into pSB2K3. Each part was cloned with its bracketing restriction sites surrounded by standard BioBricks restriction sites [Knight, 2002]. We used site directed mutagenesis on parts 6, 7, 14 and 20 to introduce the sites U1, U2, U3 and U4 respectively. Site directed mutagenesis on part 20 failed.

A single Eco0109I restriction site was removed by site directed mutagenesis from the vector pUB119BHB carrying scaffold Fragment 4. Part 15 was subsequently cloned into this modified vector. Scaffold Fragment 4 was then transferred by cloning into pREB. The following parts were serially cloned into this vector: 7, 8, 12, 13, 14, 16, 18, 20, 22 and 23. The now-populated scaffold Fragment 4 was digested with NheI and BclI and purified.

Parts 5 and 6 were cloned into pUB119BHB carrying scaffold Fragment 3. This populated Fragment 3 was subsequently used for *in vitro* assembly of a construct spanning from the left end of T7 to part 7. *In vitro* assembly of this construct began with digestion of wild-type T7 genomic DNA with AseI and isolating the 388bp left end fragment and ligating this to scaffold Fragment 2. The correct ligation product was selected by PCR. The mutation in part 3 (A1) was then fixed by PCR ligation by a two-step process. First primers with the corrected sequence for part 3 were used to amplify the two halves of the construct to the left and right of part 3. A subsequent PCR ligation

was carried out to join these two constructs. Scaffold Fragment 3 was then added to the above left-end construct once again by PCR ligation as described above. The mutation in part 4 (A2, A3 and R0.3) was repaired similarly to the mutation in part 3. The right most primer used to amplify the entire construct contained an MluI site on the tail that was then used to ligate on a copy of part 7. The ligation product was again selected by PCR. This populated left-end construct was then digested by NheI and purified.

The right arm of a BclI digestion of wild-type T7 genomic DNA was then isolated and ligated to the populated left end construct and the populated Scaffold Fragment 4. The three-way ligation product was then transfected into IJ1127 [*Materials and Methods*]. Plaques were used to create lysates and DNA was purified and digested to screen for desired clones [*Materials and Methods*].

Part 11 was then cloned into the rebuilt section using the same method of three-way ligation followed by transfection. Cloning of part 9 required the *in vitro* assembly of a construct that spanned part 6 through part 9 because RsrII(D9L/R) cuts wild-type T7 elsewhere. The construct was created by amplifying the region spanning part 5 through part 12 of the refactored genome by PCR. The PCR product was then digested with RsrII and ligated to part 9. The correct ligation product was selected by PCR with a primer on the right end that contained a SacII site in the tail. This PCR product was then digested with SacI and SacII and cloned into the phage as described above. Lastly, the part 10 was cloned into the SacII site of the phage.

Chapter 4.4.3.2. Beta

We constructed section *beta* using a process similar to that used with *alpha.* A scaffold with all restriction sites as well as part 26 was made by Klenow extension of overlapping primers. The product was digested with BstBI and cloned into pREB. The following parts were then cloned into this vector: 23, 24, 27, 28, 30, 31 and 32. Part 32 (gene *3.8*) had to be cloned as a truncation since we were unable to clone the full-length part, probably due to the previously reported toxicity of gene *3.8* product [Molineux IJ, personal communication]. The truncated version of part 32 still included the BglII site to

allow for assembly of section *beta* into a phage. Parts 25 and 29, which were also previously reported to be toxic, were assembled *in vitro*. To insert part 25, we amplified a region spanning part 23 through part 27 by PCR. This fragment was then digested with BsiWI and part 25 was ligated to each of these fragments separately and selected for by PCR. These two PCR products were then digested with DraIII, a restriction site internal to part 25, ligated and then selected by PCR. The overall fragment was then digested with BclI and MluI, purified, and ligated to wild-type fragments on the left and right. The same method was used to insert part 29 by using the part 29 internal restriction site EcoO109I and then digesting this overall fragment with MluI and BglII for cloning into a phage. Lastly, these two phage genomes were digested with MluI and the left fragment of the genome containing the refactored region spanning part 23 to 27 was ligated to the right fragment of the genome containing the refactored region spanning from part 27 to 32.

Chapter 4.4.3.3. Synthesis & Construction Errors

Differences between the designed and constructed sections *alpha* and *beta* are detailed in Tables 7-1 and 7-2.

Chapter 4.4.4. Materials and Methods

Chapter 4.4.4.1. Strains

E. coli

BL21: B *hsdS Gal-*

BR3: B *rpoC-E2258K*

D1210: HB101 *lacIq*

DH5alpha: ϕ80*lacZΔM15 Δ(lacZYA-argF) U169 endA1 recA1 hsdR17 (rk-, mk+) thi-1 gyrA96 relA1 phoA*

DH10B: *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 deoR recA1 araD139* Δ (*ara leu*)7697 *galU galK rpsL endA1 nupG*

IJ1126: *E. coli* K-12 *recB21 recC22 sbcA5 endA gal thi* Su+ Δ(*mcrC-mrr*)*102::*Tn*10*

IJ1127: IJ1126 *lacUV5 lacZ::*T7 *gene1*-Knr

Phage

T7+, wild-type bacteriophage T7, was a gift of Dr. Ian Molineux (UT Austin)

Chapter 4.4.4.2. Media Recipes

L-broth or LB Medium (Luria-Bertani Medium) [Sambrook & Russell, 2001]

10 g Bacto-tryptone

5 g yeast extract

10 g NaCl

distilled water up to 1 L

1.5% T-agar [Garcia, 1996]

10 g Bacto-Tryptone

5 g NaCl

15 g Bacto-agar

distilled water up to 1 L

0.7% T-agar [Garcia, 1996]

10 g Bacto-Tryptone

5 g NaCl

7 g Bacto-agar

distilled water up to 1 L

50X TAE Electrophoresis Buffer [Sambrook & Russell, 2001]

242 g Tris base

57.1 ml glacial acetic acid

100 ml 0.5 EDTA pH 8.0

distilled water up to 1 L

T7 Buffer [Garcia, 1996]

0.1 M Tris-HCl pH 7.5

1 M NaCl

1 mM EDTA pH 7.5

TES Buffer [Garcia, 1996]

50 mM NaCl

50 mM Tris-HCl pH 7.5

5mM EDTA pH 7.5

ϱ=*1.43 cesium chloride* [Garcia, 1996]

33 g cesium chloride

50 ml 10 mM Tris-HCL pH 7.5, 10mM $MgCl_2$

ϱ=*1.53 cesium chloride* [Garcia, 1996]

41 g cesium chloride

50 ml 10 mM Tris-HCL pH 7.5, 10mM $MgCl_2$

ϱ=*1.62 cesium chloride* [Garcia, 1996]

50 g cesium chloride

50 ml 10 mM Tris-HCL pH 7.5, 10mM $MgCl_2$

TE [Sambrook & Russell, 2001]

10mM Tris-Cl pH 8.0

1mM EDTA pH 8.0

Chapter 4.4.4.3. General Protocols

4.4.4.3.A Plating of T7

T7 was plated by adding various dilutions of a phage stock to 200uL of saturated BL21 culture and 3mL of molten (46°C) 0.7% T-agar and pouring the mixed contents onto 1.5% T-agar plates. Plaques appeared after 3-5 hours of incubation at 37°C.

4.4.4.3.B Isolation of T7 Genomic DNA From Crude Cell Lysates

T7 genomic DNA was isolated according to the protocol described in Rene Garcia's dissertation [Garcia, 1996], reproduced here:

1. Grow 40 ml of permissive cells to a density of 108-109 cells/ml at 37°C in a rotary shaking water bath. Inoculate the cells with a drop from a master phage stock. Continue to shake cells in the water bath at 37°C until the culture clarifies.
2. Add NaCl to a final concentration of 1 molar. Centrifuge the lysate at 10,000 rpm for 10 min. Discard the cellular debris, and centrifuge the lysate at 24,000 rpm for 90 min in a SW28 rotor (Beckman).
3. Discard the supernatant, and add 1 ml of T7 buffer or TES buffer to the phage pellet.
4. Let the pellet site at 4°C for at least 5 hours. Resuspend the pellet, and quickly spin down the cellular debris. Discard the pellet.
5. To the supernatant, add 0.5 ml of 50 mM Tris-HCl, pH 8 saturated phenol and gently mix the sample until an emulsion forms. Quickly microfuge the sample to separate the layers. Carefully remove the aqueous layer without disturbing the organic layer. Phenol extract with 0.5 ml 50 mM Tris-HCl, pH 8 saturated phenol one more time.
6. To the aqueous layer, add 0.5 ml of 50mM Tris-HCl, pH 8 saturated phenol: chloroform: isoamyl alcohol (25:34:1 by volume) mixture, and gently mix the sample until an emulsion forms. Quickly microfuge the sample to separate layers. Carefully remove the aqueous layer without disturbing the organic layer. Phenol: chloroform: isoamyl alcohol and extract the aqueous layer one more time.
7. Add 3 times volume of 95% ethanol alcohol to the sample. A fibrous precipitate should form. Spin down the precipitate, remove the supernatant, and wash the pellet with ethanol. Dry the pellet. Dissolve the pellet in 500 uL of water or TES buffer. There should be about 10^9 molecules of phage DNA/uL. Store the DNA at -20°C.

4.4.4.3.C Purification of T7 Particles via CsCl-gradient Centrifugation

T7 particles were purified by cesium chloride gradient centrifugation according to Garcia's protocol [Garcia, 1996]. The protocol is reproduced here:

1. Grow 100ml of permissive cells to a density of 10^8 to 10^9 cells/ml at 37°C in a rotary shaking water bath. Inoculate the cells with a drop from a master phage stock. Continue to shake cells in the water bath at 37°C until culture clarifies. [NOTE – As a standard laboratory protocol, T7 stocks have always been propagated at 30°C; however, at this temperature cultures infected with (A1, A2, A3)- T7 mutants take longer to clarify that those infected with (A1, A2, A3)+ phages or with mutants that eject their DNA faster. At 37°C the differences in lysis periods are not as pronounced. Stocks of (A1, A2, A3)- T7 mutants are propagated at 37°C to decrease the growth disadvantage of spontaneous arising mutants that eject their DNA faster. For constancy (A1, A2, A3)+ phages are also grown at this higher temperature.]
2. Add NaCl to the lysate to make the final concentration 1 molar. Centrifuge the lysate at 10,000 rpm for 10 min, Discard the cellular debris, and add 10 grams polyethylene glycol (PEG) m.w. 8000 (10% w/v) to the supernatant. Gently stir the mixture until the PEG has totally dissolved. Keep lysate on ice for 1 hour.
3. Pellet the phage at 5,000 rpm for 15 min. Decant the supernatant, and very gently resuspend the pellet in 3.5 ml of T7 buffer. Centrifuge the lysate at 5,000 rpm for 10 min, and keep the supernatant.
4. Pour a cesium chloride step gradient: ass .5 ml of cesium chloride with a density of 1.6 to the bottom of a centrifuge tube that fits in a SW 40.1 rotor. Gently layer 0.5 ml of cesium chloride ρ=1.5 onto the ρ=1.6 layer. Finally ass 0.5 ml of cesium chloride ρ=1.4 onto the ρ=1.5 layer.
5. Gently layer the phage supernatant onto the cesium chloride step gradient. Centrifuge the phage in a SW 50.1 rotor at 30,000 rpm for 2 to 3 hours. The phage will band at the ρ=1.5 layer.

6. Remove the phage band from the side of the tube with a syringe.
7. Remove the cesium chloride by dialysis against 0.5 to 1 liter of T7 buffer at 4°C.

4.4.4.3.D Purification of T7 Genomic DNA From CsCl-gradient Purified Phage Particles

T7 particles were purified by cesium chloride gradient centrifugation (above). The DNA was then purified by subsequent rounds of phenol and phenol:chloroform extraction as follows: pH7.8 phenol [Sambrook & Russell, 2001] was added in a 1:1 volume ratio to the sample and the tube was inverted to mix the aqueous and organic phases. The mixture was centrifuged for 10 minutes at 13,000g. The aqueous layer was removed and subjected to an additional round of phenol extraction. This resulting aqueous layer was added in a 1:1 volume ratio to pH7.8 phenol:chloroform:isoamyl alcohol (25:24:1) [Sambrook & Russell. 2001], mixed and centrifuged for 5 minutes. This extraction step was repeated again. The DNA was precipitated by adding a 10% sample volume of 3M sodium acetate and 2-5 sample volumes of cold (4°C) absolute ethanol. The samples were mixed and incubated at -80°C for 1 hour. The DNA was then pelleted by centrifugation for 30 minutes at 13,000g and at 4°C. The DNA pellet was washed once with 80% ethanol, dried and resuspended in TE buffer.

4.4.4.3.E Purification of Restriction Enzyme Digested Fragments

All restriction enzyme digestions were carried out according to the manufacturer's directions. All fragments smaller than 10kb were purified using Qiaquick gel extraction kit (Qiagen). All fragments larger than 10kb were purified by electro-elution as follows: 20ug of digested product was preincubated with 1uL of a 1000X solution of SYBR Gold (Molecular probes) for 15 minutes. 200ng of DNA was loaded into each well of a 0.5% TAE agarose gel and electrophoreised at 1-1.5V/cm and at 4°C for 16-20 hours. Agarose blocks containing desired restriction fragments were excised under UV transillumination and loaded into a dialysis bag (3500 MWCO Snakeskin dialysis tubing, Pierce) containing 1X TAE. Fragments were electro-eluted from the agarose blocks for 1-5 hours at 5V/cm. After the completion of electro-elution was confirmed by UV

visualization, the electric field was reversed for 1 minute to aid in elution. The liquid contents of the bag were then subjected to one round of phenol extraction to remove trace amounts of agarose and the DNA was ethanol precipitated and resuspended in TE buffer.

4.4.4.3.F Plating of Phage for Comparative Plaque Analysis

Stocks of cesium chloride purified phage were serially diluted to an appropriate titer. 50, 100 or 200uL of that dilution was mixed with 200uL of saturated BL21 culture, added to 12mL of molten (50°C) 0.7% T-agar and plated directly on Petri dishes. Plaques were allowed to grow for 5-48 hours at 30°C or 37°C.

4.4.4.3.G Measuring Phage Lysis Curves

1mL containing 2x108 cells of BL21 was infected at a MOI of 5 and 200uL of the resulting mixture was loaded per well into a 96 well ViewPlate (Packard) at 30°C. Mineral oil was layered into each well and the OD was monitored at 30°C with agitation by a Wallac Victor3 plate reader (Perkin-Elmer).

4.4.4.3.H Sequencing of DNA

All sequencing of phage DNA was performed using the dideoxy terminator method. All sequencing was performed by the MIT Biopolymers Laboratory using a Perkin Elmer Applied Biosystems Division model 377 DNA sequencer. When long regions of DNA were sequenced, primers were designed at 500-800bp intervals to both sense and antisense strands. All reported sequence represents at least two separate sequence runs with no intervening ambiguities.

4.4.4.3.I Template preparation of phage genomic DNA for sequencing

For phage sequencing, only full length packaged genomic DNA (described above) was used as template.

4.4.4.3.J Template preparation of cloned parts for sequencing

The preparation of sequencing template for cloned parts was done using Qiaprep spin Miniprep Kit (Qiagen). When the quantity of purified plasmid was insufficient for

sequencing, a subsequent Templiphi (Amersham) reaction was used to amplify the sequencing template.

4.4.4.3.K Template preparation of in-vitro constructs for sequencing

In-vitro constructs were amplified using PCR, gel purified and used as template in a sequencing reaction.

Chapter 4.4.4.4. Genome Design and Sequence Analysis Tools

Restriction Site Distribution

All design and annotation of DNA constructs was done using Vector NTI (InforMax). All restriction analyses were performed with Vector NTI (InforMax), NEBcutter [Vincze et al, 2003], and REBASE [Roberts et al, 2005]. A perl script was written to search for sites within coding regions where restriction sites could be introduced by silent mutation [http://web.mit.edu/endy/www/software/cuts/].

Sequencing Analysis and Contig Assembly

Sequence analysis and contig assembly was done with AlignX and Contig Express (InforMax).

Chapter 4.4.4.5. Genome Construction Protocols

4.4.4.5.A Oligonucleotide Synthesis

All oligonucleotides were synthesized by MWG, Invitrogen or using an ABI Model 394 DNA synthesizer (Tom Knight).

4.4.4.5.B Part Amplification

All parts were amplified by PCR using the following reaction mixture: 5uL 10X Thermo Pol Buffer (NEB), 20pM primer1, 20pM primer2, 3-30ng T7 genomic DNA, 1unit Vent polymerase (NEB), 10uM each dNTP and water to 50uL. The mixture was

thermocycled (MJ Research PTC-200) as follows: 95°C for 2 minutes, 25-35 cycles of 95°C for 30 seconds, 50°C-60°C for 30 seconds, 72°C for 1-5 minutes, 72°C for 10 minutes.

4.4.4.5.C Part Cloning

All parts and vectors (0.1 - 50pmoles) were restriction enzyme digested according to the manufacturers' directions (NEB, Fermentas). Parts and vectors were then purified by gel electrophoresis (0.5 - 2% TAE agarose gel, 3 - 8 V/cm) and extracted with Qiaquick gel extraction kit (Qiagen). Ligation reactions using T4 DNA ligase (NEB) were carried out in a 3:1 part:vector molar ratio according to the manufacturer's directions. Ligation products were dialyzed on nitrocellulose membranes (Millipore) against 1000X volume of water for 30 minutes. Ligation products were transformed by electroporation using 1800V across a 1mm gap (Bio-Rad Gene Pulser Xcell) and plated on the appropriate medium. Screening for clones was performed by colony PCR with the following protocol: Colonies were picked and diluted in 100uL of water. 1uL of that cell suspension was added to 1uL 10X Thermo Pol Buffer (NEB), 4pM primer1, 4pM primer2, 0.5U Taq Polymerase (NEB), 2uM each dNTP and water to 10uL. This mixture was thermocycled as follows: 95°C for 6 minutes, 25-35 cycles of 95°C for 30 seconds, 54°C for 30 seconds, 72°C for 1-5 minutes, 72°C for 10 minutes.

4.4.4.5.D Site Directed Mutagenesis of Parts

Site specific changes were performed on the cloned parts using QuickChange Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's directions. Primers were 5' phosphorylated using Polynucleotide Kinase (NEB) according to the manufacturer's directions.

4.4.4.5.E Construction of pREB Plasmid

pREB was constructed from pSB2K3-1 [http://parts.mit.edu], a chimera of pSCANS-5 (gift of John Dunn, Brookhaven National Laboratory) and pSB1A3-1 [http://parts.mit.edu]. The multiple cloning site of pSB2K3-1 was replaced by a PstI-BstBI-BclI multiple cloning site by primer annealing and cloning. Primer duplexes were prepared

using the following steps: the reaction mixture, 100pM each primer, 2uL restriction buffer (NEB) and distilled water to 20uL was incubated as follows: 95°C for 4 minutes, 0.1°C/s ramp to 80°C, 80°C for 4 minutes, 0.1°C/s ramp to 70°C, 70°C for 4 minutes, 0.1°C/s ramp to 60°C, 60°C for 4 minutes, 0.1°C/s ramp to 50°C, 50°C for 4 minutes, 0.1°C/s ramp to 22°C, 22°C for 10 minutes. The annealed duplexes were 5' phosphorylated using Polynucleotide Kinase (NEB). pREB was then cleaned of restriction sites using QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene) and screened by digestion.

4.4.4.5.F Construction and Cloning of the Beta Scaffold

The beta scaffold was constructed by annealing two partially overlapping primers as described above. The overhangs were then filled in using Klenow fragment (NEB) extension according to the manufacturer's directions. The extension product was digested with BstBI and cloned into pREB.

4.4.4.5.G Assembly of Section Fragments in E. coli:

Parts were cloned into the scaffold using the same cloning method as above with the addition of treating the purified cut vector with Antarctic Phosphatase (NEB) according to the manufacturer's directions and using a molar ratio of 6-10:1 insert:vector in the ligation reaction. Screening was again performed by colony PCR but included a second PCR to verify directionality via an internal primer.

4.4.4.5.H Assembly of Section Fragments in vitro

Fragments were assembled *in vitro* using both PCR ligation and traditional T4 DNA ligation. T4 DNA ligation products were subsequently selected by PCR. All amplification was carried out as described above using either Taq polymerase, Vent Polymerase or a 99:1 Taq:PfuTurbo (Stratagene) enzyme mixture. Selection of ligation products was carried out using various serial dilutions of the ligation products as template.

4.4.4.5.I Ligation of 2 or 3 DNA Fragments for Phage Transfection

$4x10^9$ molecules of each DNA fragment was ligated using T4 DNA ligase (NEB) and incubated at 16°C overnight. In certain cases, up to $4x10^{10}$ molecules of a particular DNA fragment was added to drive the reaction towards the desired outcome.

4.4.4.5.J Preparation of Competent Cells for Phage Transfection

Competent cells were prepared according to Garcia's protocol [Garcia, 1996] and allowed to rest at 4°C for 20-24 hours prior to transfection. Garcia's protocol is reproduced here:

1. Grow 20 ml of cells to a density of 5 x 10^8 cells/ml in L broth at the desired temperature.
2. Pellet the cells in a centrifuge at 5,000 rpm for 5 min. Remove the supernatant.
3. Resuspend the cell pellet in 10 ml of ice-cold 50mM CaCl2 (half volume of starting culture).
4. Incubate the cells on ice for 30 min.
5. Pellet the cells in a centrifuge at 5,000 rpm for 5 min. Remove the supernatant.
6. Resuspend the cell pellet in 2 ml of ice-cold 50 mM CaCl2 (one-tenth volume of the starting culture). The cells are ready to take up DNA.

4.4.4.5.K Transfection of the Ligation Products

All pipette tips were pre-chilled at -20°C, molten 0.7% T-agar was kept at 46°C and 1.5% T-agar plates were equilibrated to room temperature. The ligation mixture was added to 200uL of cold (4°C) competent cells and incubated in an ice bath for 30 minutes. The mixture was then added to 2.5mL of molten (46°C) 0.7% T-agar, gently mixed for 10 seconds by manual agitation and poured onto a 1.5% T-agar plate. The plates were then incubated at 37°C for 3-5 hours.

Chapter 5. Future & Conclusions

Chapter 5.1. Simulation

I have built a new simulator called Tabasco that allows simulation of gene expression at a single-molecule, single base-pair resolution by encoding logical rules of gene expression directly into the simulator. Using these rules, Tabasco does not have to track or encode every possible state, just those states that are accessed during the course of a simulation. Efforts for building simulators similar to Tabasco will continue to be useful for systems where the number of possible states is much larger than the number of molecules that transition between those states. For example, the biosynthesis of the large polysaccharide chains (glycosaminoglycans) on proteoglycans is a multi-step pathway leading to proteins with large structural diversity [Esko, 1999]. The production of glycosaminoglycans (GAGs) is difficult to explore using traditional simulation methods because the number of possible species that may arise during sugar production are large. Using logical rules of how enzymes recognize, modify, and extend GAGs *in vivo*, we can simulate the evolution of individual GAGs in order to explore the mechanisms of proteoglycan formation. In collaboration with Jonathan Behr and Tarak Upadhyaya, we are constructing a simulator based on Tabasco to simulate GAG formation.

Chapter 5.2. Modeling

I constructed a best-faith model for gene expression during the infection of bacteriophage T7 using the Tabasco simulator. Together with Heather Keller, we found that the previous discrepancies between simulations and measurements of class I and II protein synthesis rates are accounted for by the previously uncharacterized degradation of mRNA. The new models of T7 gene expression are better and more detailed, but we still need to address several points of uncertainty before we can expect accurate model-based predictions.

First, we need to understand mechanisms that lead to the newly characterized mRNA degradation. While we have characterized degradation rates on the natural genome, the question remains as to what factors determine the stability of a particular message. If we move a gene from one position to the next, can we reasonably predict the stability of the gene's mRNA? Second, our understanding of RNA polymerase processivity and interactions on the genome is limited. Recent studies have explored such processes in greater detail, and may help to improve future models [Zhou & Martin, 2006; Epshtein & Nudler, 2003]. Third, more work on the functioning of individual components in the context of a larger genome is needed in order for new genome architectures to reliably work according to expectation. Fourth, we must reevaluate our assumptions that gene expression and DNA replication are independent processes. In the process of measurements of mRNA levels during infection, we observed transcription late in infection of a region of DNA that most likely was due to genome concatemer formation during DNA replication. If transcription and replication are concurrent, the expected increase of transcription rates of class III mRNA could lead to better correspondence between the measurements and simulations [§4]. Finally, the model would benefit from system-wide absolute measurements of protein levels and relative measurements of the strengths of the ribosome binding sites, the latter of which are currently being made by Heather Keller.

Construction of a better model will allow exploration of how genome designs are (or are not) optimized to solve challenges T7 is faced with. For example, an individual T7 particle, suspended in solution, lacks information detailing the size, growth state, and genotype of its future host cell. At the time of infection, the number of other T7 particles that are simultaneously infecting the host is uncertain. However, we know that wild-type T7 can produce near-identical growth curves at multiplicities of infection (MOI) from 1 to ~20 [Endy, personal communication]. We also know that wild-type T7 plaques continue to grow as host cells begin to enter stationary phase and that T7 can infect ~1/10th volume minicells [Ponta et al., 1977; Libby et al., 1984; Shepherd et al., 2001]. While other phage (e.g., lambda) use variation in host cell state and multiplicity of infection to trigger developmental decisions (e.g., lysis or lysogeny) [Ptashne, 2004], T7 appears able to maintain its consistent lifestyle in spite of environmental uncertainty.

One mechanism for controlling system response to uncertainty in MOI and cell volume would be to use negative feedback [Becskei & Serrano, 2000; Rosenfeld et al., 2002] to regulate the activity of the *E. coli* and T7 RNA polymerases, wherein the rates of polymerase inhibition would depend on the rates of synthesis for the early and middle T7 proteins. A cell infected at a high MOI would inactivate the host RNA polymerase faster, producing less phage protein on a per-phage-infecting basis. Similarly, a phage that infected a large cell would be able to synthesize more of the early proteins needed to take advantage of increased host resources.

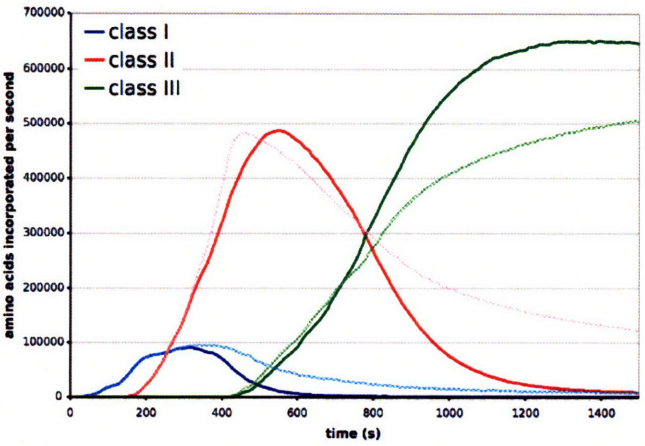


Figure 5-1. Simulated effects of the loss of negative feedback regulation on phage resource allocation during infection. The amino acid incorporation rate for the class I (blue), class II (red), and class III (green) proteins are plotted as a function of infection time with (dark) and without (light) transcriptional feedback mechanisms encoded in the simulation. Class I and class II proteins show sustained expression at the expense of class III protein production when the transcriptional feedback mechanisms are not present.

Both early and middle T7 proteins are known to inhibit the *E. coli* and T7 RNA polymerases [§3.2.3]. The standard model for T7 infection is that gp0.7, gp2 and gp3.5 are used to control transitions in T7 gene expression, from early to middle to late classes, and to help initiate DNA replication [Figure 5-1; Studier & Dunn, 1983]. The relative positioning of these genes on the genome play an important role in determining the extent of the feedback inhibition because the timing and level of gene expression is in essence controlled by the gene location. An accurate model for T7 gene expression would allow us to explore the extent that the natural T7 genome is optimized to handle such uncertainty.

Chapter 5.3. Refactoring and T7.2

The design and synthesis of T7.1 afforded us a first experience at design and construction of an engineered T7 genome. For example, the *design* of section *alpha* took six person-months to complete. We were not entirely confident that section *alpha*, with ~400 base pairs changed or added, would be viable. So, during the design phase, we were careful to try to exclude every possible change that might impact phage development. We did this by minimizing changes of variables that we were aware of, from genetic element secondary structure to untranslated mRNA regions. When we discovered that section *alpha* was viable, we began design of section *beta*, which took two-person weeks. We then subsequently designed the rest of the T7.1 genome in three-person days. Some of the reduction in design times arose via simple optimization of techniques, but another change was due to our knowledge of the types and extent of changes that could be made.

However, the resulting T7.1 genome is more constrained by our initial uncertainty than driven by our primary design goal – to construct a genetic system that is a physical instance of our understanding of a biological system that we believe, to first approximation, to understand. I have designed and planned construction of a second-generation synthetic T7 genome, which I designate T7.2, in order to improve our understanding of the functional genetic elements that contribute to phage gene expression. The design of T7.2 makes good use of our initial experience with designing and building T7.1 [§2]. For example, we now know that the wild-type T7 genome can tolerate many simultaneous engineered changes without gross impact to phage viability.

Five goals drove the design of T7.2; the first four goals revisited or extended those used in the design of T7.1. First, the T7.2 genome *only* includes elements that are thought to contribute to phage gene expression. Moving beyond our design of T7.1, I actively erased or deleted elements of unknown function. Second, the T7.2 genome design does not include any functions that might be encoded via the physical coupling of multiple genetic elements. Third, the design of T7.2 enables unique and selection-independent manipulation of each genetic element via restriction enzymes. Fourth, for practical reasons, the design of T7.2 is designed to encode a viable bacteriophage. Fifth, to attempt to make our modeling of gene expression easier, I used standard synthetic elements in place of the natural elements that regulate transcription and translation.

Taken together, the design of T7.2 should specify a genome that is simpler to model and manipulate, in which we have a putative function for each base pair of DNA involved in phage gene expression. Thus, I hypothesize that T7.2 will also encode a dynamic system that is easier to model and interact with, relative to the wild type.

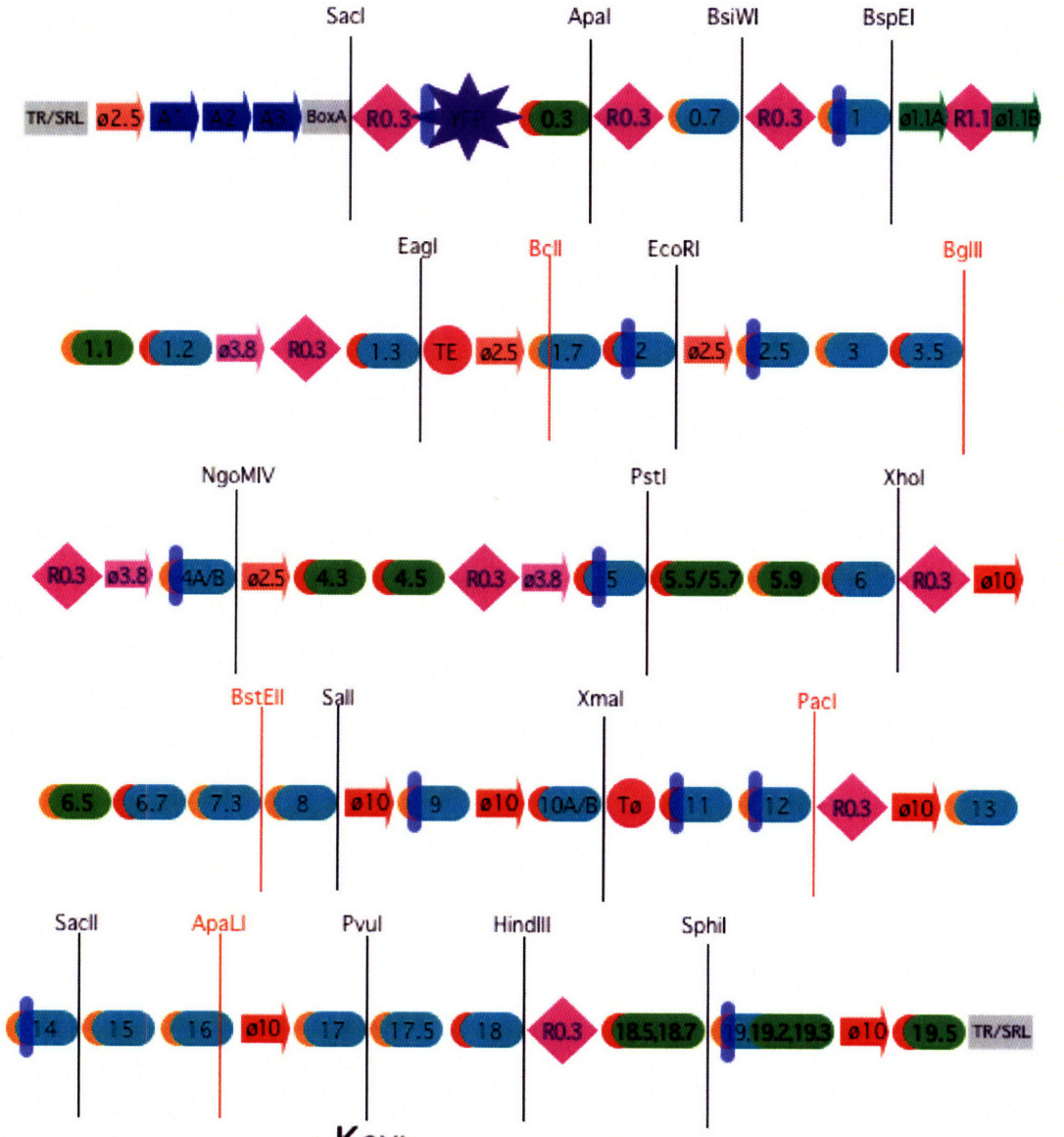
Chapter 5.3.1. T7.2 Design Specification

The design of the T7.2 genome is a component-based design. I defined and specified each component, added them to the Registry of Standard Biology Parts (http://parts.mit.edu), and compiled the individual components to construct a design draft of the genome. Part numbers indexed in the Registry take the form BBaZ0XXX. After the initial draft, I did global optimizations such as restriction site additions, RBS-CDS secondary structure removal, and addition of elements to aid in measurement of system function to construct a draft sequence of the T7.2 genome (BBaZ0506). The following sections describe the considerations made for each step of the process.

Chapter 5.3.1.1. Coding Domains

There are 57 annotated genes on the T7 genome encoding 60 proteins. Many of the natural T7 genes are not known to contribute to phage development in the set of conditions in which I wish to assay them, and they confound systems-level analysis. The

Figure 5-2. T7.2 Design (next page). In the T7.2 design, I defined and concatenated individual parts to arrive at the design. The labeled genetic elements include protein coding regions (light blue & green lozenges), strong and medium strength ribosome binding sites (orange & red arcs before coding domains), the three major *E. coli* promoters (blue arrows), two standardized classes of T7 weak promoters (orange & pink arrows), standardized T7 strong promoters (red arrows), standardized RNase III recognition sites (pink diamonds), transcription terminators (red circles), a fluorescent reporter construct (purple stars), and other regions of importance (gray). Sites where I removed RNA secondary structure across the RBS-gene interface are labeled by verticle blue lozenges. The genome will be constructed in sections to make hybrid phage at labeled natural T7 single cut sites (red lines). Additional single-cut restriction sites were used throughout the design to aid in further manipulations (black lines).



Key:

Genes to Remove

0.4 0.5 0.6A/B 1.4

1.5 1.6 1.8 2.8

3.8 4.1 4.2 4.7

5.3 6.3 7 7.7

CDS that will be in all iterations of T7.2

CDS to remove iteratively

ø10 Std. T7 strong promoter

ø2.5 Std. T7 weak binding promoter

ø3.8 Std. T7 weak binding & processivity promoter

Std strong ribosome binding site

Std medium ribosome binding site

Shuffled RBS/gene to remove 2° structure

following genes are not conserved across other T7 relatives and encode no known function during T7 development: *0.4*, *0.5*, *0.6A/B*, *1.4*, *1.5*, *1.6*, *1.8*, *2.8*, *3.8*, *4.1*, *4.2*, *4.7*, *5.3*, *6.3*, *7*, *7.7*. I did not include these genes in the design of T7.2. I also included many genes that will eventually be removed, but am including to be cautious. These genes mostly show evolutionary conservation or anecdotal evidence for important functions. I included the genes in the design, but have made them easier to remove in the future. These genes are *0.3*, *1.1*, *4.3*, *4.5*, *5.5-5.7*, *5.7*, *5.9*, *6.5*, *18.5*, *18.7*, *19.2*, *19.3*, *19.5*.

Chapter 5.3.1.2. Ribosome Binding Sites

Each gene has a corresponding putative ribosome binding sites (RBS) that controls translation initiation. I standardized the RBS of these genes to reduce the parameters necessary to represent the system, and possibly to reduce any unknown functions of translation coupling. I chose three RBS to standardize from that were measured by Heather Keller to have a varied range of expression rates. I assigned one of these RBS to each coding domain based on both knowledge of the amounts of total protein production necessary for phage function, as well as computational analysis of the strength of existing ribosome binding sites. The computational analysis assigned predicted strengths [Table 6-3] of the RBS based on their sequence through a published algorithm [Barrick et al., 1994]. I standardized the natural sites to three ribosome binding sites (strong, medium, and weak) that were characterized experimentally by Heather Keller. After the design was completed, I found the weak RBS only mapped to genes that were not included in the T7.2 design. Thus I use only two standardized RBS - the strong RBS based on the gene *5* (BBaZ0261), and an exogenous RBS (BBaZ0262; Weiss, 2001). The assignments of the RBS are shown in Figure 5-2.

Chapter 5.3.1.3. Host Promoters

I encoded only the three strong host promoters (A1, A2, A3). I removed the other annotated promoters by virtue of not including genes they were contained in, or by point

mutations intended to obliterate function. The edits are annotated at http://parts.mit.edu. The following is the listing of promoters that were removed:

B: Located mostly in gene *0.5*, which is not encoded

C: Only -35 region is included within coding domain of gene *0.7*; -10 region and start are not included in T7.2 design.

E: Strong -10 region mutated, -35 region not included

S1: -35 and -10 regions edited to remove promoter

S2: -35 region edited, -10 region already not optimal

S3: -35 and -10 regions edited to remove promoter

S4: -35 and -10 regions edited to remove promoter

S5: Located within gene 5.3 which is not included

S6: -35 and -10 regions edited to remove promoter

F1: Only -35 region is included within coding domain of gene *6.5*; n 10 region and start are not included in T7.2 design.

F2: -35 region mutated, -10 region already not optimal

Chapter 5.3.1.4. Phage Promoters

I standardized the phage promoters that I encoded into the genome. In our models, I split the promoters into three class; weak binding and processivity, weak processivity, or strong promoters [§3.6.1.2]. The natural T7 promoters on the T7 genome are listed in Table 3-4, along with their classification. I used the *ø2.5*, *ø3.8*, and *ø10* promoters as the standard for the weak processivity (BBaZ0253), weak processivity and binding (BBaZ0252), and the strong promoters (BBaZ0251), respectively. I chose these three promoters they were used in past detailed kinetic studies. Applying a conservative approach, each promoter occupies 35 bp, more than the necessary and sufficient 23 bp. Finally, as a consequence of removing genes, sometimes two T7 promoters would become directly adjacent. In those cases, I removed one of the two T7 promoters. The final distribution of T7 promoters is shown in the final design diagram [Figure 5-2].

Chapter 5.3.1.5. Miscellaneous elements

I treated the primary origin of replication, and the promoters that are contained within it (*ø1.1A* and *ø1.1B*), as a separate part in order to prevent disturbances (BBaZ0291). In addition, I treated the left end (BBaZ0292; 377 bp) and right end (BBaZ0293; 399 bp) of the genome as individual parts due to their large repeats and importance in DNA replication and packaging. Also, I kept the definitions for the transcriptional terminators as they were in T7.1. TE (BBaZ0271) and Tø (BBaZ0272), while the CJ terminator remained unaltered in the right end part. Finally, I specified addition of a fluorescent reporter, Venus YFP, into the genome [Nagai et al., 2002].

Chapter 5.3.1.6. RBS - CDS Interface

To avoid possible effects of introduced secondary structure at the interface between the standardized RBS and the coding domains that could interfere with expression, Alex Mallet and I analyzed and minimized secondary structure for many genes. Alex designed software to evaluate secondary structure effects, and developed a minimization procedure to reduce the secondary structure across the part interface [Mallet, 2006]. Briefly, we used the RNAfold algorithm [Hofacker et al., 1994] to evaluate free energy of the possible secondary structures of the 20 RBS with the first 13 codons of the CDS. Structures that had significant secondary structure across the interface (>-9 kCal/mol) were marked to be modified. Those structures that were changed are labeled on Figure 5-2. For each of the genes to be altered, we chose three alternative codons for each amino acid residue, and calculated secondary structures of all possible combinations of the codons. The search reduced secondary structure pairing significantly. I used the minimum free energy structure for the coding domains in the T7.2 design.

Chapter 5.3.1.7. Restriction Enzyme Cut Sites.

In the T7.1 design, I flanked each part with restriction enzyme recognition sites to allow easy part manipulation. However, I was limited by the number of independent recognition sites, and thus many of the restriction sites cut more than once in different sections of the T7.1 genome. We had expected to be able to clone substantial portions of each genome, but the sections proved to be fairly toxic. I found that having single cut sites spaced farther apart would be better for future manipulations. After the draft initial T7.2 design was complete, I added restriction cut sites that cut only once on the genome approximately every 1000 bp [Figure 5-2].

Chapter 5.3.2. T7.2 Construction Plans

The manual construction of T7.1 proved to be very tedious. Thus, I explored automated ways to construct T7 sections. Commercial synthesis companies have become somewhat adept at building DNA the size of T7 sections. However, most companies rely on cloning in *E. coli* in order to produce their DNA. I worked with Codon Devices on *in vitro* methods to construct the genomes. Codon Devices constructed the first 8000 bp of the T7.2 genome in 9 ~1000 bp fragments. Efforts on combining these fragments together is still ongoing, and is now beyond the scope of this thesis.

Chapter 5.4. Conclusions

In this thesis, I worked to construct accurate and biophysically realistic models of gene expression for a natural biological system, bacteriophage T7. I constructed more detailed simulation tools [§2], and used them to make new, more detailed models of bacteriophage T7 gene expression [§3]. Heather Keller then made system-wide measurements of T7 mRNA levels, and I showed that the inability of the previous models to account for class I and II protein synthesis attenuation late in infection can be accounted for by mRNA degradation. However, the models still lack sufficient information to provide predictable gene expression in T7 variants containing arbitrary

genetic rearrangements. For example, if we move a gene from one position to another, we would not have a mechanistic model for how the rate of mRNA degradation would change.

We can continue to improve computational models by better characterizing individual components, and compare them to system-wide measurements to analyze the validity of models. Differences between the models and measurements can lead to further understanding, and point towards future work. We can hope that in the future, our models will progress to a point where they are realistic representations of the natural phage, and that model-based predictions of arbitrary perturbations can be trusted.

I also embarked on a complementary approach to the process of trying to understand natural systems. Rather than making models of T7 evermore detailed, I decided to construct synthetic phage that are closer physical representations of our models. Leon Chan and I began, by designing and constructing a portion of a new phage, T7.1, where we define and insulate genetic elements eliminating genetic overlaps that we assigned no function to in our models [§4]. The resulting phage had similar gross characteristics to T7 and the genome of T7.1 is now easier to model.

I have also designed a new phage, T7.2, that goes beyond simply specifying a genome that is easier to model, to a phage where infection is easier to model [§5.3]. For example, in our models, I assume the measured kinetics of one T7 strong promoter are the same for all the strong T7 promoters. We could further study the phage promoters in their individual contexts to test the validity of the assumption. Alternatively, we can standardize the relevant sequences to match the model more accurately. Thus, we don't necessarily care about the natural T7 genome per se, we care about how individual elements in a genome can interact to produce the dynamic system that leads to development of a replicating and productive phage.

Construction of synthetic biological systems in support of understanding and analyzing natural biological systems can serve a number of purposes. *First*, recapitulation of observable functions provides a powerful test of our understanding of how system components interact to form a functioning whole [Sprinzak & Elowitz, 2005]. Differences between models used to design synthetic systems and the synthetic systems themselves point to gaps in our understanding of the governing components and

interactions [Chan et al., 2005]. Likewise, differences between synthetic and natural systems can lead to insight into what other functions the natural biological systems might serve. *Second*, evolving surrogate synthetic systems can act as a tool to understand how the individual parts of the natural system contribute to system fitness, as well as provide a platform to understand how evolutionary processes can optimize system performance. *Third*, constructing more defined, measurable, and manipulable developmental systems provides a foundation for the rational engineering of novel self-replicating, self-assembling systems [Knight, 2005].

Chapter 6. References

Abelson H, Sussman GJ, Sussman J (1996) *Structure and Interpretation of Computer Programs*, 2nd edition. MIT Press, Cambridge, Massachusetts, USA

Ackers GK, Johnson AD, Shea MA (1982) A quantitative model for gene regulation by λ phage repressor. *Proc Natl Acad Sci USA* **79:**1129

Adelman K, et. al. (2002) Single molecule analysis of RNA polymerase elongation revelas uniform kinetic behavior. *Proc Natl Acad Sci USA* **99:**13538

Adhya S, Gottesman M (1982) Promoter occlusion: Transcription through a promoter may inhibit its activity. *Cell* **29:**939

Aho A-C, Donner K, Hyden C, Larsen LO, Reuter T (1988) Low retinal noise in animals with low body temperature allows high visual sensitivity. *Nature* **334:**348

Anand VS, Patel SS (2006) Transient state kinetics of transcription elongation by t7 RNA polymerase. *J Biol Chem* **281:**35677

Andrews SS, Bray D (2004) Stochastic simulation of chemical reactions with spatial resolution and single molecule detail. *Phys Biol* **1:**137

Arkin A, Ross J, McAdams HH (1998) Stochastic kinetic analysis of developmental pathway bifurcation in phage lambda-infected Escherichia coli cells. *Genetics* **149:**1633

Bai L, Santangelo TJ, Wang MD (2006) Single-molecule analysis of RNA polymerase transcription. *Annu Rev Biophys Biomol Struct* **35:**343

Bandwar RP, Tang GQ, Patel SS (2006). Sequential release of promoter contacts during transcription initiation to elongation transition. *J Mol Biol* **360:**466

Bandwar RP & Patel SS (2002). The energetics of consensus promoter opening by T7 RNA polymerase. *J Mol Biol* **324:**63

Bandwar RP, Jia Y, Stano NM, Patel SS (2002) Kinetic and thermodynamic basis of promoter strength: multiple steps of transcription initiation by T7 RNA polymerase are modulated by the promoter sequence. *Biochemistry* **41:**3586

Barrick D et al., (1994) Quantitative analysis of ribosome binding sites in E.coli. *Nucleic Acids Res* **22:**1287

Becskei A, Serrano L (2000) Engineering stability in gene networks by autoregulation. *Nature* **405:**590

Block SM, Segall JE, Berg HC (1982) Impulse responses in bacterial chemotaxis. *Cell* **31:**215

Boothroyd JC, Hayward RS (1979) New genes and promoters suggested by the DNA sequence near the end of the coliphage T7 early operon. *Nucleic Acids Res* **7:**1931

Bremer H, Dennis PP (1996) Modulation of chemical composition and other parameters of the cell by growth rate. In *Escherichia coli and Salmonella: Cellular and Molecular Biology,* 2nd edition. eds. Neidhardt FC. ASM Press, Washington D.C.

Bremer H, Yuan D (1968) RNA chain growth-rate in Escherichia coli. *J Mol Biol* **38:**163

Brenner S, Jacob F, Meselson M (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190:**576

Brunovskis I, Summers WC (1972) The process of infection with coliphage T7. VI. A phage gene controlling shutoff of host RNA synthesis. *Virology* **50:**322

Briat JF, Chamberlin MJ (1984) Identification and characterization of a new transcriptional termination factor from Escherichia coli. *Proc Natl Acad Sci USA* **81:**7373

Buchholtz F, Schneider FW (1987) Computer simulation of T3/T7 phage infection using lag times. *Biophys Chem* **26:**171

Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* **25:**169

Cao Y, Gillespie DT, Petzold LR (2005) Improved leap-size selection for accelerated stochastic simulation. *J Chem Phys* **119:**8229

Carlson R (2001) Open-source Biology And Its Impact On Industry. *Spectrum, IEEE* **38:**15

Carlson R (2003) The pace and proliferation of biological technologies. *Biosecur Bioterror* **1:**203

Carter AD, McAllister WT (1981) Sequences of three class II promoters for the bacteriophage T7 RNA polymerase. *J Mol Biol* **153:**825

Cech CL, Lichy J, McClure WR (1980) Characterization of promoter containing DNA fragments based on the abortive initiation reaction of Escherichia coli RNA polymerase. *J Biol Chem* **10**:1763

Chamberlin MJ, Nierman WC, Wiggs J, Neff N (1979) A quantitative assay for bacterial RNA polymerases. *J Biol Chem* **254**:10061

Chan LY, Kosuri S, Endy D (2005) Refactoring bacteriophage T7. *Mol Syst Biol* **1**:2005.0018

Chock PB, Stadtman ER (1977) Superiority of Interconvertible Enzyme Cascades in Metabolic Regulation: Analysis of Multicyclic Systems. *Proc Natl Acad Sci USA* **74**:2766

Court DL, Sawitzke JA, Thomason LC (2002) Genetic engineering using homologous recombination. *Annu Rev Genet* **36**:361

Crampton N, Bonass WA, Kirkham J, Rivetti C, Thomson NH (2006) Collision events between RNA polymerases in convergent transcription studied by atomic force microscopy. *Nucleic Acids Res* **34**:5416

Dalbow DG, Young R (1975) Synthesis time of beta-galactosidase in Escherichia coli B/r as a function of growth rate. *Biochem J* **150**:13

Davidson EH (1999) A view from the genome: spatial control of transcription in sea urchin development. *Curr Opin Genet Dev* **9**:530

Davis BL (1946) Del rigor en la ciencia. *Los Anales de Buenos Aires*, **año 1**(3). See also http://en.wikipedia.org/wiki/On_Exactitude_in_Science

Davis RW, Hyman RW (1970) Physical Locations of the in vitro RNA Initiation Site and Termination Sites of T7 DNA. *Cold Spring Harb Symp Quant Biol* **35**:269

Dayton CJ, Prosen DE, Parker KL, Cech CL (1984) Kinetic measurements of Escherichia coli RNA polymerase association with bacteriophage T7 early promoters. *J Biol Chem* **259**:1616

de Smit MH, van Duin J (2003) Translational standby sites: how ribosomes may deal with the rapid folding kinetics of mRNA. *J Mol Biol* **331**:737

Delbrück M (1946) Bacterial viruses or bacteriophages. *Biol Rev Camb Philos Soc* **21**:30

Delius H, Westphal H, Axelrod N (1973) Length measurements of RNA synthesized in vitro by Escherichia coli RNA polymerase. *J Mol Biol* **74**:677

Demerec M, Fano U (1945) Bacteriophage-resistant mutants in Escherichia coli. *Genetics* **30**:119

Donachie WD, Robinson A (1987) Cell division: parameter values and the process, p. 1578. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* ed by Neidhardt et al. American Society for Microbiology, Washington D.C., USA

Dressler D, Wolfson J, Magazin M (1972) Initiation and reinitiation of DNA synthesis during replication of bacteriophage T7. *Proc Natl Acad Sci U S A* **69:**998

Dubendorff JW, Studier FW (1991) Creation of a T7 autogene. Cloning and expression of the gene for bacteriophage T7 RNA polymerase under control of its cognate promoter. *J Mol Biol* **219:**61

Dunn JJ, Studier FW (1973) T7 early RNAs are generated by site-specific cleavages. *Proc Natl Acad Sci U S A* **70:**1559

Dunn JJ, Studier FW (1980) The transcription termination site at the end of the early region of bacteriophage T7 DNA. *Nucleic Acids Res* **8:**2119

Dunn JJ, Studier FW (1981) Nucleotide sequence from the genetic left end of bacteriophage T7 DNA to the beginning of gene 4. *J Mol Biol* **148:**303

Dunn JJ, Studier FW (1983) Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J Mol Biol* **166:**477

Elowitz MB, Levine AJ, Siggia ED, Swain PS (2002) Stochastic gene expression in a single cell. *Science* **297**:1183

Endy D, Kong D, Yin J (1997) Intracellular kinetics of a growing virus: A genetically structured simulation for bacteriophage T7. *Biotechnol Bioeng* **55:**375

Endy D, You L, Yin J, Molineux IJ (2000) Computation, prediction, and experimental tests of fitness for bacteriophage T7 mutants with permuted genomes. *Proc Natl Acad Sci U S A* **97**:5375

Endy D, Brent R (2001) Modelling Cellular Behavior. *Nature* **409:**391-395

Epshtein V, Nudler E (2003) Cooperation between RNA polymerase molecules in transcription elongation. *Science* **300**:801-805

Epshtein V, Toulme F, Rahmouni AR, Borukhov S, Nudler E (2003) Transcription through the roadblocks: the role of RNA polymerase cooperation. *EMBO J* **22:**4719

Esko JD (1999) Biosynthesis, Metabolism, and Function: Proteoglycans and Glycosaminoglycans. in *Essentials of Glycobiology*, eds. Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Forde NR, et. al. (2002) Using mechanical force to probe the mechanism of pausing and arrest during continuous elongation by Escherichia coli RNA polymerase. *Proc Natl Acad Sci USA* **99**:11682

Fowler M, Beck K, Brant J, Opdyke W, Roberts D (1999) *Refactoring: Improving the Design of Existing Code.* Addison-Wesley Professional, Boston, Massachusetts, USA

Garcia LR (1996) *Characterization of bacteriophage T7 DNA entry into Escherichia coli.* Ph.D. Dissertation. The University of Texas, Austin, Texas, USA

Garcia LR, Molineux IJ (1995) Rate of translocation of bacteriophage T7 DNA across the membranes of Escherichia coli. *J Bacteriol* **177:**4066-4076

Gibson MA, Bruck J (2000) Efficient exact stochastic simulation of chemical systems with many species and many channels. *J Phys Chem A* **104:**1876

Gilbert W (1976) in *RNA Polymerase* (Losic R, Chamberlin M, eds.) pp. 193-205, Cold Spring Harbor Press, Cold Spring Harbor.

Gillespie DT (1976) A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. *J Comp Phys* **22**:403

Gillespie DT (1977) Exact stochastic simulation of coupled chemical reactions. *J Phys Chem* **81:**2340

Gillespie DT, Petzold LR (2003) Improved leap-size selection for accelerated stochastic simulation. *J Chem Phys* **119:**8229

Goldbeter A, Koshland DE (1981) An Amplified Sensitivity Arising from Covalent Modification in Biological Systems. *Proc Natl Acad Sci USA* **78:**6840

Golomb M, Chamberlin M (1974a) Characterization of T7-specific ribonucleic acid polymerase. IV. Resolution of the major in vitro transcripts by gel electrophoresis. *J Biol Chem* **249:**2858

Golomb M, Chamberlin M (1974b) A preliminary map of the major transcription units read by T7 RNA polymerase on the T7 and T3 bacteriophage chromosomes. *Proc Natl Acad Sci U S A* **71:**760

Greive SJ, von Hippel PH (2005) Thinking quantitatively about transcriptional regulation. *Nat Rev Mol Cell Biol* **6:**221

Grunberg-Manago, M (1999) Messenger RNA stability and its role in control of gene expression in bacteria and phages. *Annu Rev Genet* **33:**193

Hausmann R, Gomez B (1967) Amber mutants of bacteriophages T3 and T7 defective in phage-directed deoxyribonucleic acid synthesis. *J Virol* **1:**779

Hausmann R, LaRue K (1969) Variations in sedimentation patterns among deoxyribonucleic acids synthesized after infection of Escherichia coli by different amber mutants of bacteriophage T7. *J Virol* **3:**278

Hercules K, Jovanovich S, Sauerbrier W (1976) Early gene expression in bacteriophage T7. I. In vivo synthesis, inactivation, and translational utilization of early mRNA's. *J Virol* **17:**642

Hesselbach BA, Nakada D (1977a) I protein: bacteriophage T7-coded inhibitor of Escherichia coli RNA polymerase. *J Virol* **24:**746

Hesselbach BA, Nakada D (1977b) "Host shutoff" function of bacteriophage T7: involvement of T7 gene 2 and gene 0.7 in the inactivation of Escherichia coli RNA polymerase. *J Virol* **24:**736

Hesselbach BA, Nakada D (1975) Inactive complex formation between E. coli RNA polymerase and inhibitor protein purified from T7 phage infected cells. *Nature* **258:**354

Hofacker LL et al (1994) Fast Folding and Comparison of RNA Secondary Structures. *Monatshefte f. Chemie* **125:**167

Horowitz H, Platt T (1982) Regulation of transcription from tandem and convergent promoters. *Nucleic Acids Res* **10:**5447

Hüttenhofer A, Noller HF (1994) Footprinting mRNA-ribosome complexes with chemical probes. **13:**3892

Ikeda RA (1992) The efficiency of promoter clearance distinguishes T7 class II and class III promoters. *J Biol Chem* **267:**11322

Ikeda RA, Bailey PA (1992) Inhibition of T7 RNA polymerase by T7 lysozyme in vitro. *J Biol Chem* **267:**20153

Ikeda RA, Warshamana GS, Chang LL (1992) In vivo and in vitro activities of point mutants of the bacteriophage T7 RNA polymerase promoter. *Biochemistry* **31:**9073

Ikemura T (1981) Correlation between the abundance of Escherichia coli transfer RNAs and the occurrence of the respective codons in its protein genes: a proposal for a synonymous codon choice that is optimal for the E. coli translational system. *J Mol Biol* **151:**389

Jacob F, Monod J (1961) On the Regulation of Gene Activity. *Cold Spring Harb Symp Quant Biol* **26:**193

Jacob F (1977) Evolution and Tinkering. *Science* **196:**1161

Jia Y, Kumar A, Patel SS (1996) Equilibrium and stopped-flow kinetic studies of interaction between T7 RNA polymerase and its promoters measured by protein and 2-aminopurine fluorescence changes. *J Biol Chem* **271:**30451

Jia Y, Patel SS (1997) Kinetic mechanism of transcription initation by bacteriophage T7 RNA polymerase. *Biochemistry* **36:**4223

Johnston DE, McClure WR (1976) in *RNA Polymerase* (Losic R, Chamberlin M, eds.) pp. 413-428, Cold Spring Harbor Press, Cold Spring Harbor.

Johnson RS, Chester RE (1998) Stopped-flow kinetic analysis of the interaction of Escherichia coli RNA polymerase with the bacteriophage T7 A1 promoter. *J Mol Biol* **283:**353

Johnson ZI, Chisholm SW (2004) Properties of overlapping genes are conserved across microbial genomes. *Genome Res* **14:**2268

Kaern M, Elston TC, Blake WJ, Collins JJ (2005) Stochasticity in gene expression: from theories to phenotypes. *Nat Rev Genet* **6:**451

Kauffman SA (1969) Metabolic stability and epigensis in randomly constructed genetic nets. *J Theoret Biol* **22:**437

Kassavetis GA, Chamberlin MJ (1979) Mapping of class II promoter sites utilized in vitro by T7-specific RNA polymerase on bacteriophage T7 DNA. *J Virol* **29:**196-208

Kelly TJ Jr, Thomas CA Jr (1969) An intermediate in the replication of bacteriophage T7 DNA molecules. *J Mol Biol* **44:**459

Kemp P, Garcia LR, Molineux IJ (2005) Changes in bacteriophage T7 virion structure at the initiation of infection. *Virology* **340:**307

Kennel D (2002) Processing Endoribonucleases and mRNA degradation in bacteria. *J Bacteriol* **184:**4645

Kennell D, Riezman H (1977) Transcription and translation initiation frequencies of the Escherichia coli lac operon. **114:**1

Kirschner MW (2005) The meaning of systems biology. *Cell* **20:**503

Knight T (2002) Idempotent Vector Design for Standard Assembly of Biobricks. *MIT Synthetic Biology Working Group Technical Report 0* [http://web.mit.edu/synbio/release/docs/biobricks.pdf]

Knuth DE (1997) *The Art of Computer Programming*, 3rd edition. Addison-Wesley, Reading, Massachusetts USA. Volume 2, Section 3.2.1.

Kodumal SJ, Patel KG, Reid R, Menzella HG, Welch M, Santi DV (2003) Total synthesis of long DNA sequences: synthesis of a contiguous 32-kb polyketide synthase gene cluster. *Proc Natl Acad Sci U S A* **101:**15573

Korzheva N et al. (2000) A structural model of transcription elongation. *Science* **289:**619

Koza JR, Keane MA, Streeter MJ, Mydlowec W, Yu J, Lanza G (2003) *Genetic Programming IV: Routine Human-Competitive Machine Intelligence.* Kluwer Academic Publishers, Dordrecht, Netherlands

Kumar A, Patel SS (1997) Inhibition of T7 RNA polymerase: transcription initiation and transition from initiation to elongation are inhibited by T7 lysozyme via a ternary complex with RNA polymerase and promoter DNA. *Biochemistry* **36:**13954

Kushner SR (2002) mRNA Decay in Eschericia coli comes of age. *J Bacteriol* **184:**4658

Libby RT, Shaw JE, Reeve JN (1984) Expression of coliphage T7 in aging anucleate minicells of Escherichia coli. *Mech Ageing Dev* **27:**197

Liu C, Martin CT (2002) Promoter clearance by T7 RNA polymerase. Initial bubble collapse and transcript dissociation monitored by base analog fluorescence. *J Biol Chem* **277:**13

Lok L, Brent R (2005) Automatic generation of cellular reaction networks with Moleculizer 1.0. *Nat Biotechnol* **23:**131

Lopez PJ, Guillerez J, Sousa R, Dreyfus M (1997) The low processivity of T7 RNA polymerase over the initially transcribed sequence can limit productive initiation in vivo. *J Mol Biol* **269:**41

Lopez PJ, Iost I, Dreyfus M (1994) The use of a tRNA as a transcriptional reporter: the T7 late promoter is extremely efficient in Escherichia coli but its transcripts are poorly expressed. *Nucleic Acids Res* **22:**1186

Luria SE, Delbrück M (1943) Mutations of Bacteria from Virus Sensitivity to Virus Sensitivity. *Genetics* **28:**491

Lyakhov DL et al. (1997) Mutant bacteriophage T7 RNA polymerases with altered termination properties. *J Mol Biol* **269:**28

Macdonald LE, Durbin RK, Dunn JJ, McAllister WT (1994) Characterization of two types of termination signal for bacteriophage T7 RNA polymerase. *J Mol Biol* **238:**145

Macdonald LE, Zhou Y, McAllister WT (1993) Termination and slippage by bacteriophage T7 RNA polymerase. *J Mol Biol* **232:**1030

Mallet A (2006) *Analysis of Targeted and Combinatorial Approaches to T7 Genome Generation.* M.A. Dissertation, Massachusetts Institute of Technology, Cambridge, Massachusetts USA.

Manor H, Goodman D, Stent GS (1969) RNA chain growth rates in Escherichia coli. *J Mol Biol* **39:**1

Marchand I, Nicholson AW, Dreyfus M (2001) Bacteriophage T7 protein kinase phosphorylates RNase E and stabilizes mRNAs synthesized by T7 RNA polymerase. *Mol Microbiol* **42:**767

Marrs BL, Yanofsky C (1971) Host and bacteriophage specific messenger RNA degradation in T7-infected Escherichia coli. *Nat New Biol* **234:**168

Masamune Y, Frenkel GD, Richardson CC (1971) A mutant of bacteriophage T7 deficient in polynucleotide ligase. *J Biol Chem* **246:**6874

Mayer JE, Schweiger M (1983) RNase III is positively regulated by T7 protein kinase. *J Biol Chem* **258:**5340

McAdams HH, Arkin A, (1997) Stochastic mechanisms in gene expression. *Proc Natl Acad Sci USA* **94:**814

McAllister WT, McCarron RJ (1977) Hybridization of the in vitro products of bacteriophage T7 RNA polymerase to restriction fragments of T7 DNA. *Virology* **82:**288

McAllister WT, Morris C, Rosenberg AH, Studier FW (1981) Utilization of bacteriophage T7 late promoters in recombinant plasmids during infection. *J Mol Biol* **153:**527

McAllister WT, Wu HL (1978) Regulation of transcription of the late genes of bacteriophage T7. *Proc Natl Acad Sci U S A* **75:**804

McClure WR (1983) DNA determinants of promoter selectivity in E. coli. *Cold Spring Harb Symp Quant Biol* **47:**477

Mettetal JT, Muzzey D, Pedraza JM, Ozbudak EM, van Oudenaarden A (2006) Predicting stochastic gene expression dynamics in single cells. *Proc Natl Acad Sci USA* **103:**7304

Michalewicz J, Nicholson AW (1992) Molecular cloning and expression of the bacteriophage T7 0.7(protein kinase) gene. *Virology* **186:**452

Minkley EG, Pribnow D (1973) Transcription of the early region of bacteriophage T7: selective initiation with dinucleotides. *J Mol Biol* **77:**255

Moffatt BA, Studier FW (1987) T7 lysozyme inhibits transcription by T7 RNA polymerase. *Cell* **49:**221

Moffatt BA, Studier FW (1988) Entry of bacteriophage T7 DNA into the cell and escape from host restriction. *J Bacteriol* **170:**2095

Molineux IJ (2001) No syringes please, ejection of phage T7 DNA from the virion is enzyme driven. *Mol Microbiol* **40:**1

Molineux IJ (2005) The T7 Group. In *The Bacteriophages,* Calendar RL (ed) Ch 20. Oxford: Oxford University Press

Morton-Firth CJ, Bray D (1998) Predicting temporal fluctuations in an intracellular signalling pathway. *J Theor Biol* **192:**117

Müler-Hill B (1996) *The lac Operon: a short history of a genetic paradigm.* de Gruyter, New York

Nagai T et al. (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat Biotechnol* **20:**87

Nechaev S, Severinov K (2003) Bacteriophage-induced modifications of host RNA polymerase. *Annu Rev Microbiol* **57:**301

Nechaev S, Severinov K (1999) Inhibition of Escherichia coli RNA polymerase by bacteriophage T& gene 2 protein. *J Mol Biol* **289:**815

Niles EG, Condit RC (1975) Translational Mapping of Bacteriophage T7 RNAs synthesized in vitro by purified T7 RNA polymerase. *J Mol Biol* **98:**57

Nomura T, Fujita N, Ishihama A (1985) Promoter selectivity of E. coli RNA polymerase: analysis of the promoter system of convergently-transcribed dnaQ-rnh genes. *Nucleic Acids Res* **13:**7647

Nudler E, Avetissova E, Markovtsov V, Goldfarb A (1996) Transcription processivity: protein-DNA interactions holding together the elongation complex. *Science* **273:**211

Nudler E, Gusarov I, Avetissova E, Kozlov M, Goldfarb A (1998) Spatial organization of transcription elongation complex in Escherichia coli. *Science* **281:**424

Oakley JL, Coleman JE (1977) Structure of a promoter for T7 RNA polymerase. *Proc Natl Acad Sci U S A* **74:**4266

Olson ER, Flamm EL, Friedman DI (1982) Analysis of nutR: a region of phage lambda required for antitermination of transcription. *Cell* **31:**61

Osterman HL, Coleman JE (1981) T7 ribonucleic acid polymerase-promoter interactions. *Biochemistry* **20:**4884

Ozbudak EM, Thattai M, Kurtser I, Grossman AD, van Oudenaarden A (2002) Regulation of noise in the expression of a single gene. *Nat Genet* **31:**69

Panayotatos N, Wells RD (1979) Recognition and initiation site for four late promoters of phage T7 is a 22-base pair DNA sequence. *Nature* **280:**35

Paulsson J (2004) Summing up the noise in gene networks. *Nature* **427:**415

Pedraza JM, van Oudenaarden A (2005) Noise propagation in gene networks. *Science* **307:**1965

Pfennig-Yeh ML et al. (1978) Early T7 gene expression: rates of RNA synthesis and degradation, protein kinase dependent termination of transcription, and efficiency of translation. *Mol Gen Genet* **166:**127

Ponta H et al. (1977) Productive T7 infection of Escherichia coli F+ cells and anucleate minicells. *Nature* **269:**440

Press WH, Teukolsky SA, Vetterling WT, Flannery BP (1992) *Numerical Recipes in C: The Art of Scientific Computing*, 2nd edition. Cambridge University Press, Cambridge, England. pgs. 290-296

Pribnow D (1975) Nucleotide sequence of an RNA polymerase binding site at an early T7 promoter. *Proc Natl Acad Sci USA* **72:**784

Rao CV, Arkin AP (2003) Stochastic chemical kinetics and the quasi-steady-state assumption: Application to the Gillespie algorithm. *J Chem Phys* **18:**4999

Ritchie DA, Malcolm FE (1970) Heat-stable and density mutants of phages T1, T3 and T7. *J Gen Virol* **9:**35

Ritchie DA, Thomas CA Jr, MacHattie LA, Wensink PC (1967) Terminal repetition in non-permuted T3 and T7 bacteriophage DNA molecules. *J Mol Biol* **23:**365

Roberts RJ, Vincze T, Posfai J, Macelis D (2005) REBASE--restriction enzymes and DNA methyltransferases. *Nucleic Acids Res* **33:**D230

Rosa MD (1979) Four T7 RNA polymerase promoters contain an identical 23 bp sequence. *Cell* **16:**815-825

Rosa (1981a) Structure analysis of three T7 late mRNA ribosome binding sites. *J Mol Biol* **147:**55

Rosa MD (1981b) DNA sequence for the T7 RNA polymerase promoter for T7 RNA species II. *J Mol Biol* **147:**199

Rosenfeld N, Elowitz MB, Alon U (2002) Negative autoregulation speeds the response times of transcription networks. *J Mol Biol* **323:**785

Rosenfeld N, Young JW, Alon U, Swain PS, Elowitz MB (2005) Gene regulation at the single-cell level. *Science* **307:**1962

Saito H, Tabor S, Tamanoi F, Richardson CC (1980) Nucleotide sequence of the primary origin of bacteriophage T7 DNA replication: relationship to adjacent genes and regulatory elements. *Proc. Natl. Acad. Sci. USA.* **77:**3917

Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA

Samoilov MS, Arkin AP (2006) Deviant effects in molecular reaction pathways. *Nat Biotechnol* **24:**1235

Savageau, MA (1974) Optimal design of feedback control by inhibition: Steady state considerations. *J Mol Evol* **4:**139

Savageau, MA (1975) Optimal design of feedback control by inhibition: Dynamic considerations. *J Mol Evol* **5:**199

Severinova E, Severinov K (2006) Localization of the Escherichia coli RNA polymerase beta' subunit residue phosphorylated by bacteriophage T7 kinase Gp0.7. *J Bacteriol* **188:**3470

Sharp PA (2000) View of life sciences in the 21st century. *J Dermatol Sci* **24:**S1

Sharp PM, Rogers MS, McConnell DJ (1984) Selection pressures on codon usage in the complete genome of bacteriophage T7. *J Mol Evol* **21:**150

Shea MA, Ackers GK (1985) The OR control system of bacteriophage Lambda: A physical-chemical model for gene regulation. *J Mol Biol* **181:**211

Skinner GM, Baumann CG, Quinn DM, Molloy JE, Hoggett JG (2004) Promoter binding, initiation, and elongation by bacteriophage T7 RNA polymerase. A single-molecule view of the transcription cycle. *J Biol Chem* **279:**3239

Shepherd N, Dennis P, Bremer H (2001) Cytoplasmic RNA Polymerase in Escherichia coli. *J Bacteriol* **183:**2527

Simon MN, Studier FW (1973) Physical mapping of the early region of bacteriophage T7 DNA. *J Mol Biol* **79:**249

Smith HO, Hutchison CA 3rd, Pfannkoch C, Venter JC (2003) Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides. *Proc Natl Acad Sci U S A* **100:**15440

Smolke CD, Keasling JD (2002) Effect of gene location, mRNA secondary structures, and RNase sites on expression of two genes in an engineering operon. *Biotechnol Bioeng* **80:**762

Spector L, Barnum H, Bernstein HJ, Swamy N (1999) Finding a better-than-classical quantum AND/OR algorithm using genetic programming. In IEEE. *Proc. of 1999 Congress on Evolutionary Computation.* Piscataway, NJ: IEEE Press. p.2239–46. For additional examples, see: http://www.genetic-programming.com/humancompetitive.html

Sprinzak D, Elowitz MB (2005) Reconstruction of genetic circuits. *Nature* **438:**443

Stahl SJ, Chamberlin MJ (1977) An expanded transcriptional map of T7 bacteriophage. Reading of minor T7 promoter sites in vitro by Escherichia coli RNA polymerase. *J Mol Biol* **112:**577

Steitz JA (1969) Polypeptide chain initiation: nucleotide sequences of the three ribosomal binding sites in bacteriophage R17 RNA. *Nature* **224:**957

Stemmer WP, Crameri A, Ha KD, Brennan TM, Heyneker HL (1995) Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. *Gene* **164:**49

Steven AC, Serwer P, Bisher ME, Trus BL (1983) Molecular architecture of bacteriophage T7 capsid. *Virology* **124:**109

Strome S, Young ET (1978) Translational control of the expression of bacteriophage T7 gene 0.3. *J Mol Biol* **125:**75

Strome S, Young ET (1980a) Chemical and functional quantitation of gene 0.3 messenger RNA during T7 infection. *J Mol Biol* **136:**417

Strome S, Young ET (1980b) Translational discrimination against bacteriophage T7 gene 0.3 messenger RNA. *J Mol Biol* **136:**433

Studier FW (1969) The genetics and physiology of bacteriophage T7. *Virology* **39:**562

Studier FW, Maizel JV (1969) T7-directed protein synthesis. *Virology* **39:**575

Studier FW (1972) Bacteriophage T7. *Science* **176:**367

Studier FW (1973a) Genetic analysis of non-essential bacteriophage T7 genes. *J Mol Biol* **79:**227

Studier FW (1973b) Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J Mol Biol* **79:**237

Studier FW (1979) Relationships among different strains of T7 and among T7-related bacteriophages. *Virology* **95:**70

Studier FW, Rosenberg AH, Simon MN, Dunn JJ (1979) Genetic and physical mapping in the early region of bacteriophage T7 DNA. *J Mol Biol* **135:**917

Studier FW (1981) Identification and mapping of five new genes in bacteriophage T7. *J Mol Biol* **153:**493

Studier FW, Rosenberg AH (1981) Genetic and physical mapping of the late region of bacteriophage T7 DNA by use of cloned fragments of T7 DNA. *J Mol Biol* **153:**503

Studier FW, Dunn JJ (1983) Organization and expression of bacteriophage T7 DNA. *Cold Spring Harb Symp Quant Biol* **47 Pt 2:**999

Studier FW, Rosenburg AH, Dunn JJ, Debendorff JW (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol* **185:**60

Summers WC (1970) The process of infection with coliphage T7. IV. Stability of RNA in bacteriophage-infected cells. *J Mol Biol* **51:**671

Summers WC, Brunovskis I, Hyman RW (1973) The process of infection with coliphage T7. VII. Characterization and mapping of the major in vivo transcription products of the early region. *J Mol Biol* **74:**291

Tabor S, Richardson CC (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc Natl Acad Sci USA* **82:**1074

Tamanoi F, Saito H, Richardson CC (1980) Physical mapping of primary and secondary origins of bacteriophage T7 DNA replication. *Proc Natl Acad Sci U S A* **77:**2656

Thattai M, van Oudenaarden A (2001) Intrinsic noise in genetic regulatory networks. *Proc Natl Acad Sci USA* **98:**8614

Thomas R (1973) Boolean formalization of genetic control circuits. *J Theor Biol* **42:**563

Thomas R, Gathoye A, Lambert L (1976) A complex control circuit: Regulation of immunity in temperate bacteriophages. *Eur J Biochem* **71:**211

Tian J, Gong H, Sheng N, Zhou X, Gulari E, Gao X, Church G (2004) Accurate multiplex gene synthesis from programmable DNA microchips. *Nature* **432:**1050

Ujvari A, Martin CT (1996) Thermodynamic and kinetic measurements of promoter binding by T7 RNA polymerase. *Biochemistry* **35**:14574

Villemain J, Sousa R (1998) Specificity in transcriptional regulation in the absence of specific DNA binding sites: the case of T7 lysozyme. *J Mol Biol* **281:**793

Vincze T, Posfai J, Roberts RJ (2003) NEBcutter: A program to cleave DNA with restriction enzymes. *Nucleic Acids Res* **31:**3688

Walter AE, Turner DH, Kim J, MH Lyttle, Muller P, Mathews DH, Zuker M (1994) Coaxial stacking of helixes enhances binding of oligoribonucleotides and improves predictions RNA folding. *Proc Natl Acad Sci USA* **91:**9218

Watson JD (1972) Origin of concatemeric T7 DNA. *Nat New Biol* **239:**197

Weiss R (2001) *Cellular Computation and Communications using Engineered Genetic Regulatory Networks* Ph.D. Dissertation. Massachusetts Institute of Technology, Cambridge, Massachusetts USA

Wolfson J, Dressler D, Magazin M (1972) Bacteriophage T7 DNA replication: a linear replicating intermediate (gradient centrifugation-electron microscopy-E. coli-DNA partial denaturation). *Proc Natl Acad Sci U S A* **69:**499

Wong ML, Medrano JF (2005) Real-time PCR for mRNA quantitation. *Biotechniques* **39:**75

Wu T, Schwartz DC (2007) Transchip: Single-molecule detection of transcriptional elongation complexes. *Anal Biochem* **361:**31

Yamada Y, Whitaker PA, Nakada D (1974a) Functional instability of T7 early mRNA. *Nature* **248:**335

Yamada Y, Whitaker PA, Nakada D (1974b) Early to late switch in bacteriophage T7 development: functional decay of T7 early messenger RNA. *J Mol Biol* **89:**293

Yamada Y, Whitaker PA, Nakada D (1975) Chemical stability of bacteriophage T7 early mRNA. *J Virol* **16:**1683

Yarchuk O, Jacques N, Guillerez J, Dreyfus M (1992) Interdependence of translation, transcription and mRNA degradation in the lacZ gene. *J Mol Biol* **226:**581

Yarchuk O, Iost I, Dreyfus M (1991) The relation between translation and mRNA degradation in the lacZ gene. *Biochimie* **73:**1533

You L, Suthers PF, Yin J (2002) Effects of Escherichia coli physiology on growth of phage T7 in vivo and in silico. *J Bacteriol* **184:**1888

You L, Yin J (2002) Dependence of epistasis on environment and mutation severity as revealed by in silico mutagenesis of phage T7. *Genetics* **160:**1273

Yount B, Curtis KM, Baric RS (2000) Strategy for systematic assembly of large RNA and DNA genomes: transmissible gastroenteritis virus model. *J Virol* **74:**10600

Yuh CH, Davidson EH (1996) Modular cis-regulatory organization of Endo16, a gut-specific gene of the sea urchin embryo. *Development* **122:**1069

You CH, Bolouri H, Davidson EH (2001) Cis-regulatory logic in the endo16 gene: switching from a specification to a differentiation mode of control. *Development* **128:**617

Yusupova GZ, Yusupov MM, Cate JH, Noller HF (2001) The path of messenger RNA through the ribosome. *Cell* **106:**233

Zavriev SK, Shemyakin MF (1982) RNA polymerase-dependent mechanism for the stepwise T7 phage DNA transport from the virion into E. coli. *Nucleic Acids Res* **10:**1635

Zaychikov E, Denissova L, Heumann H (1995) Translocation of the Escherichia coli transcription complex observed in the registers 11 to 20: "jumping" of RNA polymerase and asymmetric expansion and contraction of the "transcription bubble". *Proc Natl Acad Sci USA* **28:**1739

Zhang X, Studier FW (1997) Mechanism of inhibition of bacteriophage T7 RNA polymerase by T7 lysozyme. *J Mol Biol* **269:**10

Zhang X, Studier FW (2004) Multiple roles of T7 RNA polymerase and T7 lysozyme during bacteriophage T7 infection. *J Mol Biol* **340:**707

Zhou Y, Martin CT (2006) Observed instability of T7 RNA polymerase elongation complexes can be dominated by collision-induced "bumping". *J Biol Chem* **34:**24441

Zillig W, et al. (1975) In vivo and in vitro phosphorylation of DNA-dependent RNA polymerase of Escherichia coli by bacteriophage-T7-induced protein kinase. *Proc Natl Acad Sci USA* **72:**2506

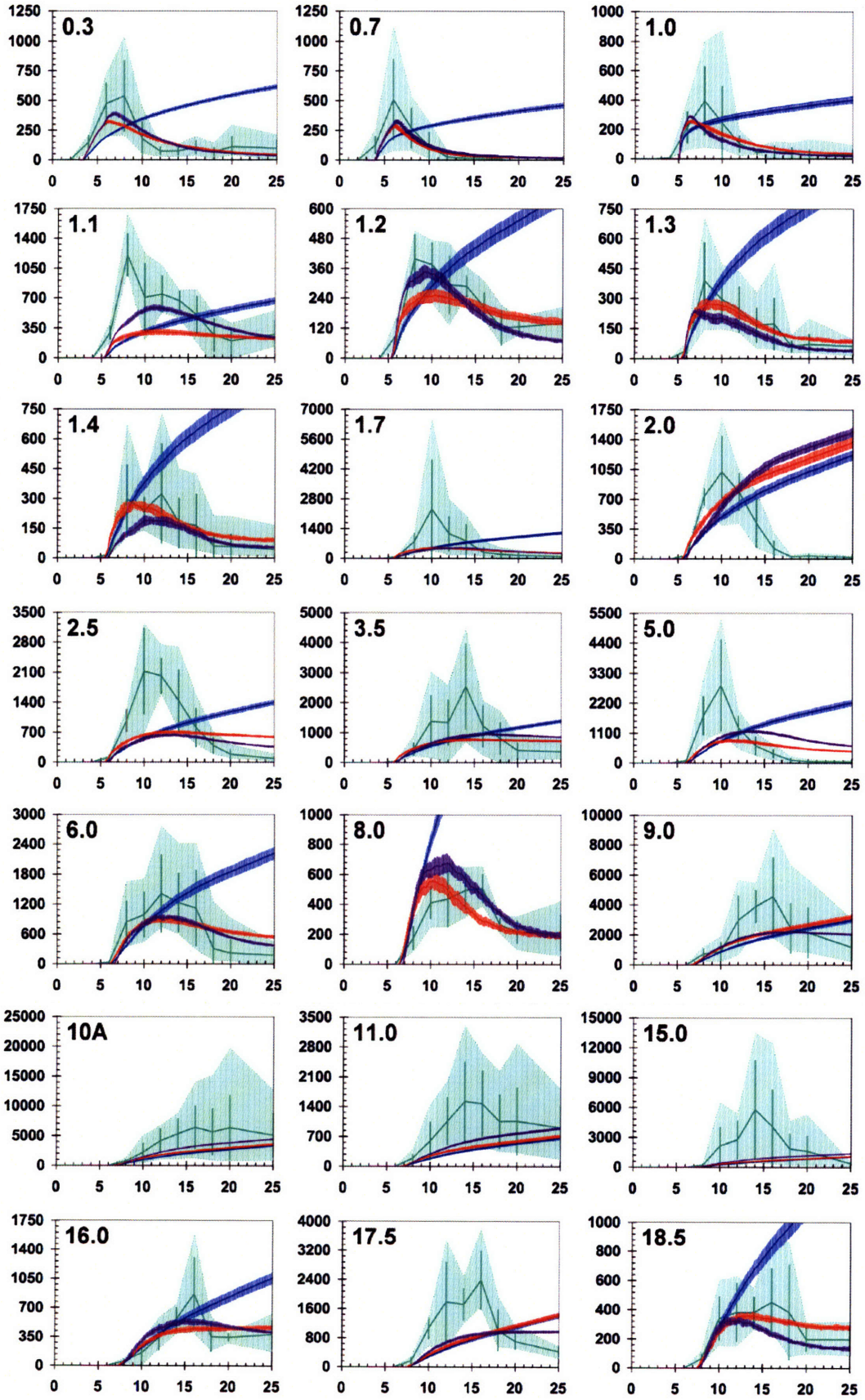
Chapter 7. Appendices

Chapter 7.1. T7 Modeling Appendix

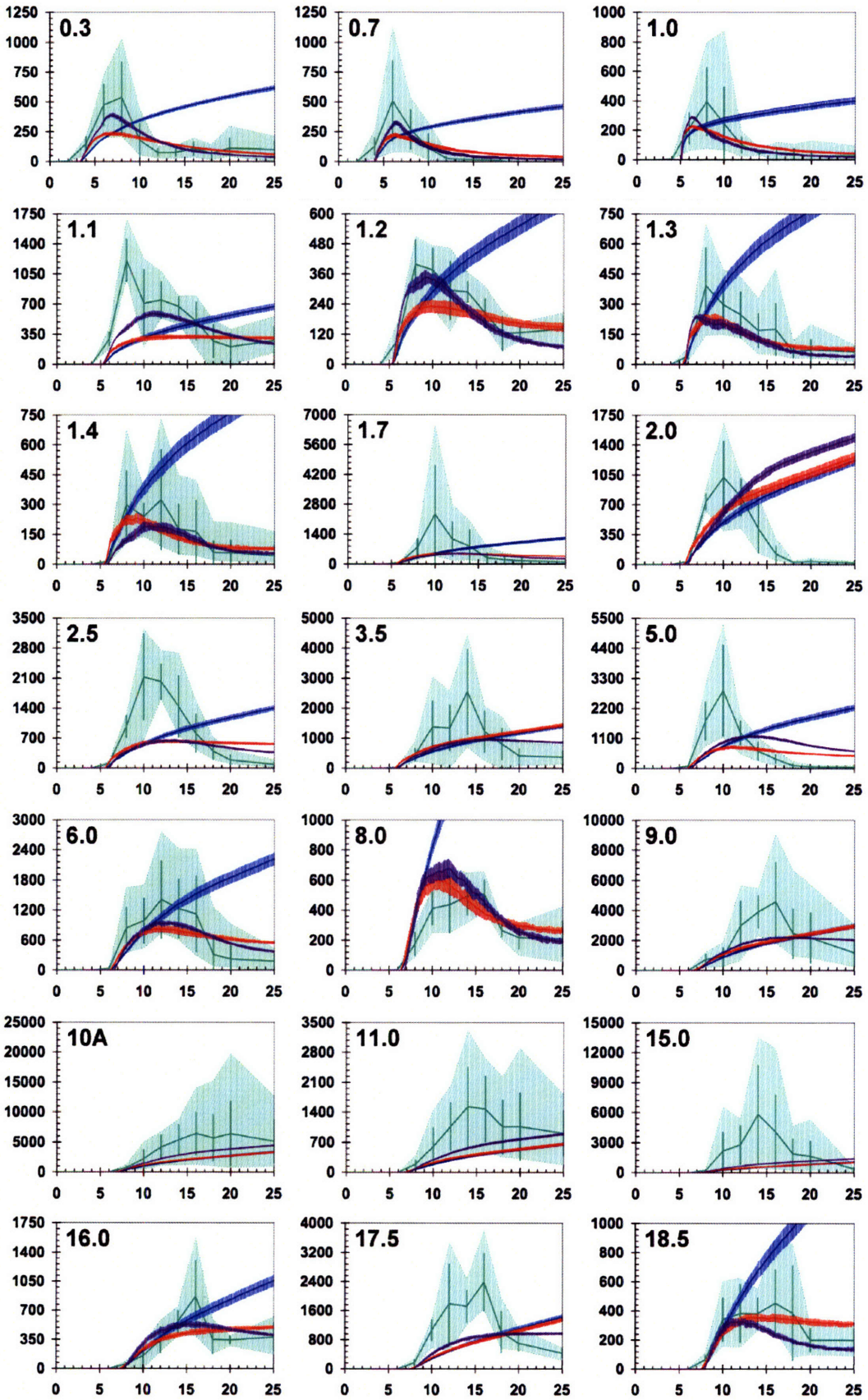
Chapter 7.1.1. Simulation and Measurements Comparisons

Figure 7-1 (following 4 pages). Real time measurements and simulation results showing absolute, gene-specific mRNA levels per cell during T7 infection. Each plot shows the measured (green) and computed (red;blue;purple) number of T7 mRNA per cell throughout infection. Measurements were made using real-time RT-PCR. For all graphs, the solid lines show the average all of all replicates (2-4 extractions per gene, each extraction measured in triplicate). Shaded regions represent the range of the measurements, with the standard deviation show by error bars. Simulation data plotted are the unfit (blue) and the best fit model (purple; traffic jam model with increased promoter strength). The red plots represent the final fitting of the other models tested: (a) Increased promoter strength; (b) Alternative degradation model; (c) DownStream Falloff model; (d) Upstream Falloff model. The width of the computed curves shows the variation (± standard deviation) from the average of 25 simulations.

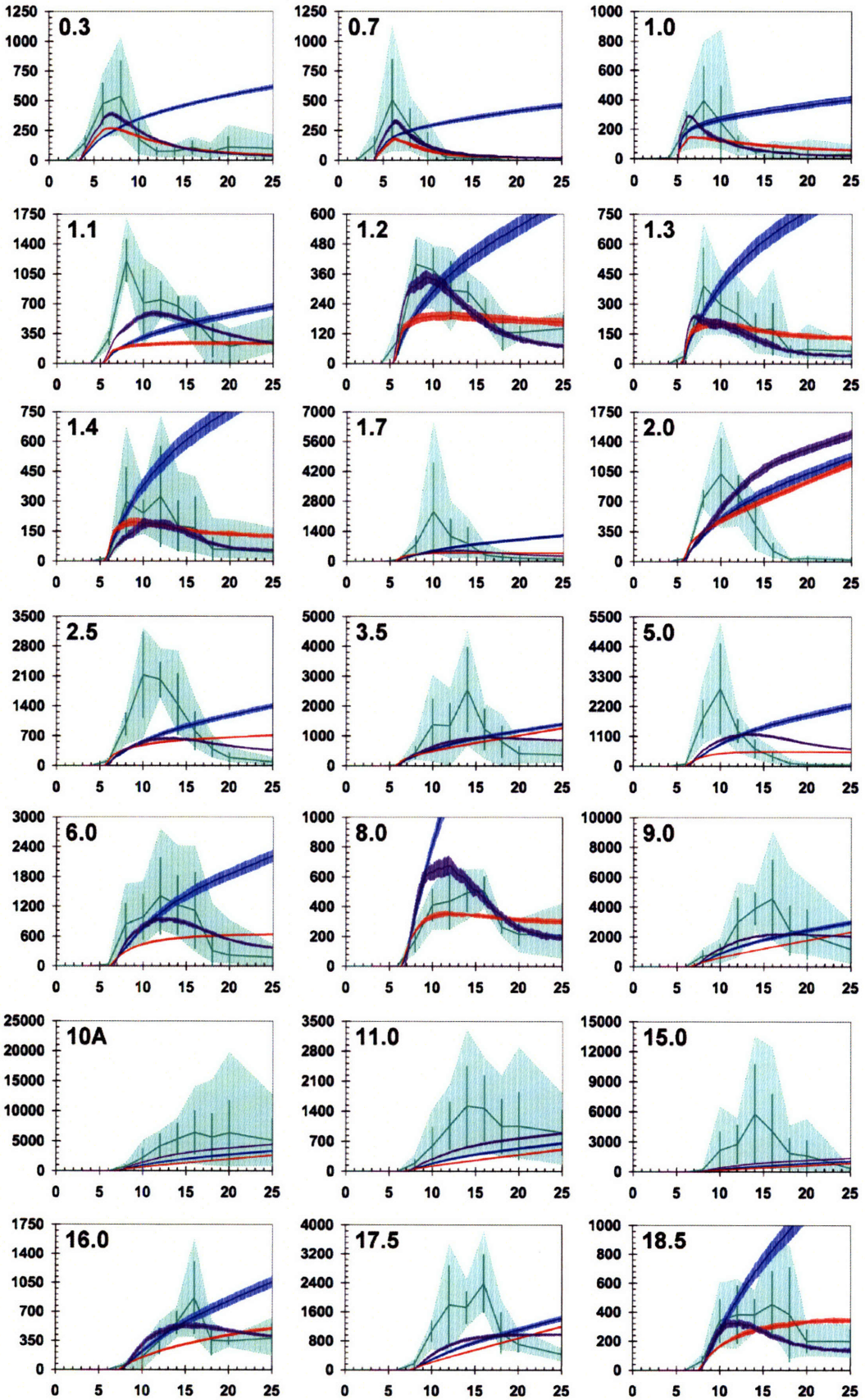
(a)



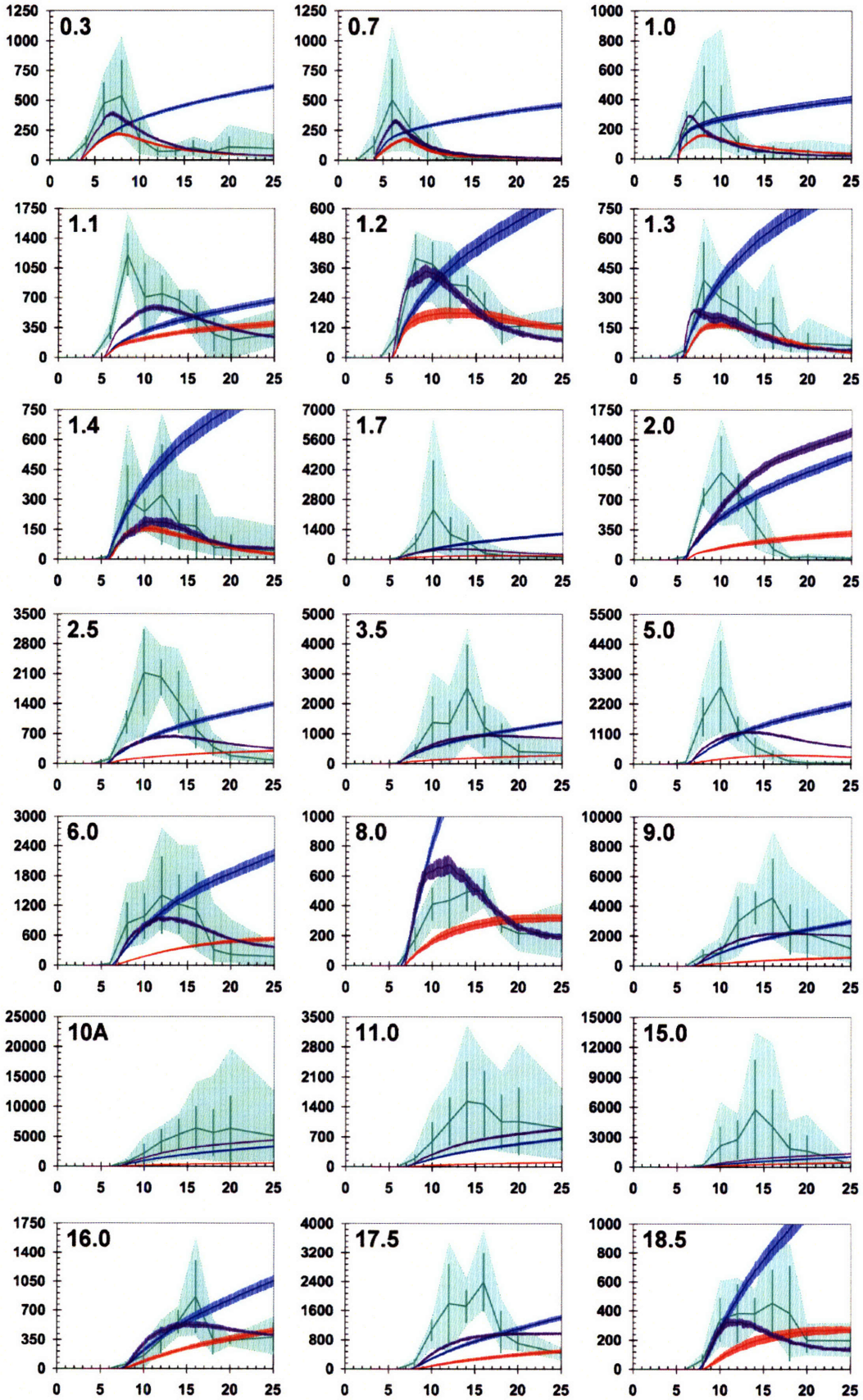
(b)



(c)



(d)



Chapter 7.1.2. Fitting Procedure Charts.

Figure 7-2. Fitting simulations of T7 mRNA levels to measured data (next 3 pages). Each table shows the optimization procedure to fit the RT-PCR mRNA measurements for the initial fit (a), the altered promoter model (b), the alternative degradation model (c), the DownStream Falloff polymerase interaction model (d), the Traffic Jam polymerase interaction model (e), and the UpStream Falloff model (f). Each row shows the simulations run to fix the mRNA levels for one particular gene, or class average. The columns represent mRNA half-lives tested in minutes. The values shown in each entry are R^2 for the particular gene and half-life being tested. A shaded box means the half-life was not tested. To begin, in row 1, an average mRNA half-life was tested for all the class I genes. The highest R^2 found (highlighted) was fixed, and I then tested and fixed the class II average half-life (row 2). After the average class III values were set, each gene was then optimized against the mRNA measurement profiles for different half-lives. After this process was complete, I show the final the final R^2 found after the last simulation in the last column. As can be seen, the R^2 were not significantly changed from the original value, meaning optimization of downstream genes did not significantly affect the fit of the upstream genes. Finally, the last row shows the average R^2 for all the individual fits.

(a)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I		0.3643	0.4078	0.4179	0.4047	0.3814	0.3579	0.3397	0.3196	0.3033						0.1229	0.4437
class II			0.4168	0.4271	0.426	0.4185	0.4107										0.4586
class III	0.2572	0.3403	0.3673	0.3612	0.354	0.3474	0.3417	0.3366	0.3359	0.335					0.327	0.3055	0.4842
0.3		0.3825	0.4142	0.417	0.4042	0.384											0.4216
0.7	0.4973	0.5323	0.5242	0.4994	0.4727												0.5297
1	0.4496	0.561	0.6364	0.6665	0.6663												0.6724
1.1				0.1414		0.154		0.1652	0.1679	0.1713	0.1723	0.1743	0.176	0.1775	0.1804	0.17	0.1755
1.2				0.2339	0.264	0.2713	0.2858	0.2758	0.259	0.2437							0.2828
1.3	0.2968	0.4712	0.5656	0.5553	0.4519	0.365	0.3026										0.5804
1.4	0.4517	0.6355	0.7417	0.6985	0.6042												0.7418
1.7				0.5338	0.5445	0.5501	0.5522	0.5558	0.5546	0.5552	0.5541	0.552				0.483	0.555
2				0.4697							0.4853	0.4867	0.4872	0.4873	0.4887	0.4951	0.4946
2.5				0.1801							0.2077	0.2064	0.2064	0.2086	0.2085	0.1792	0.2073
3.5				0.3281							0.3916	0.3972	0.4016	0.406	0.4059		0.4083
5	0.2307	0.257	0.2751	0.2831	0.2882	0.2838	0.2813	0.2756									0.287
6				0.4639	0.492	0.5042	0.5091	0.5059	0.4956								0.5159
8	0.1717	0.4609	0.5093	0.2437	0.1324												0.5355
9			0.2783										0.3932	0.3978	0.4046	0.4502	0.4529
10A			0.3359										0.4241	0.4287	0.4327	0.5148	0.5161
11			0.2899										0.3558	0.3621	0.3653	0.4189	0.4216
15			0.4305										0.4579	0.4599	0.4603	0.4773	0.4765
16			0.2579										0.4799	0.4855	0.4723	0.2677	0.4786
17.5			0.1662					0.2058	0.2093	0.2129	0.2136	0.2192	0.2183	0.2208	0.2222	0.2323	0.232
18.5		0.458	0.6367	0.7605	0.7434	0.6616	0.5722										0.7605
Total Avg																	**0.4641**

(b)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I	0.3272	0.4329	0.5077	0.4895	0.4448											0.0799	0.5699
class II	0.3641	0.4307	0.4572	0.4603	0.4508	0.4383	0.429	0.4174								0.2795	0.5195
class III			0.3549	0.3684	0.3606	0.3551	0.3532	0.3515	0.3505		0.3473		0.3442		0.3431	0.3231	0.5079
0.3		0.5296	0.5809	0.531	0.4549												0.5838
0.7	0.6053	0.6667	0.6413	0.5772	0.5146												0.6703
1	0.4493	0.6069	0.7148	0.7455	0.7336												0.7649
1.1			0.1433	0.1605	0.1731	0.1833	0.1909	0.1962	0.2056	0.205							0.1982
1.2			0.2371	0.3108	0.3993	0.4132	0.385	0.331	0.2771	0.2288							0.4370
1.3	0.346	0.6196	0.7132	0.4991	0.344												0.7652
1.4		0.7514	0.7986	0.6305	0.4506	0.3368	0.2468										0.8426
1.7		0.5144	0.5483	0.5741	0.5877	0.5974	0.5973	0.5946	0.5918	0.5922							0.5912
2		0.4628	0.4736	0.4822	0.4878	0.4924	0.4975	0.4991	0.5013	0.5044	0.5064	0.5057	0.5082	0.5091	0.5096	0.5182	0.5161
2.5	0.1406		0.186	0.2062	0.2214		0.2376		0.2466	0.246	0.2476	0.2466	0.2455		0.2391	0.1621	0.2456
3.5				0.3608	0.3842	0.4024	0.4184	0.434	0.4382	0.453	0.4575	0.4625	0.4683	0.4731	0.4715		0.4751
5				0.331	0.3356	0.3279	0.3196	0.3078	0.2958	0.2846	0.2748	0.2632	0.2565	0.2495			0.3324
6		0.3925	0.4975	0.5855	0.627	0.6387	0.6137	0.5827	0.5398								0.6336
8	0.191	0.3965	0.2699	0.1175	0.0654	0.044	0.0323										0.4022
9							0.3839	0.4006	0.4132	0.4263	0.4363	0.4452	0.4537	0.4613	0.4689	0.5123	0.5098
10A							0.4034				0.4452	0.4505	0.4588	0.4647		0.5871	0.5897
11							0.3444				0.3773	0.3809	0.3857	0.3923	0.3974	0.48	0.4791
15							0.4555				0.4644	0.4682	0.4697	0.471	0.4714	0.4902	0.4905
16					0.3924	0.4396	0.4769	0.5086	0.5209	0.5267	0.5224	0.5168	0.5099	0.4992			0.5230
17.5							0.2132				0.2314	0.2347	0.2384	0.2393	0.2435	0.2499	0.2481
18.5			0.7255	0.8322	0.7616	0.6294	0.5201										0.8212
Total Avg																	**0.5295**

(c)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I	0.2453	0.3279	0.4061	0.4505	0.4472											0.0801	0.4831
class II	0.3526	0.4022	0.4412	0.4603	0.452	0.4404	0.4362	0.4174	0.2873								0.4972
class III				0.3612	0.3634	0.3593	0.3525	0.3501	0.3473	0.346		0.3433		0.3411		0.3206	0.4903
0.3	0.261	0.3476	0.4155	0.4484	0.4409												0.4578
0.7		0.4742	0.5297	0.5405	0.5131	0.4912											0.5502
1			0.5968	0.6582	0.6977	0.6884	0.6609										0.7050
1.1								0.1836	0.1869	0.195	0.1976	0.199	0.2043	0.2035	0.2071	0.1784	0.1989
1.2				0.2512	0.3259	0.3619	0.3777	0.3503	0.3101	0.28							0.3658
1.3	0.3045	0.5153	0.6744	0.591	0.4049	0.3093	0.2183										0.6210
1.4		0.6905	0.802	0.7141	0.5077	0.3921											0.7609
1.7				0.5551	0.5708	0.5786		0.5866	0.5903	0.5872	0.5847	0.5823					0.5874
2				0.477								0.5024	0.5047	0.5038	0.5058	0.5137	0.5112
2.5				0.1903						0.2375	0.2393	0.2422	0.24	0.2366	0.2386	0.1644	0.2324
3.5				0.3453									0.4432	0.4483	0.4514	0.4618	0.4611
5				0.3078	0.3185	0.3215	0.3168	0.3111	0.3081								0.3240
6				0.4976	0.5888	0.6033	0.5896	0.5792									0.6033
8	0.1603	0.3526	0.4084	0.2206	0.1166			0.0413									0.4176
9								0.3618					0.4149	0.4215	0.4256	0.4944	0.4908
10A								0.3949					0.4315	0.4379	0.4427	0.5495	0.5534
11								0.3359					0.3657	0.3706	0.3751	0.4542	0.4520
15								0.4515					0.4614	0.4624	0.4637	0.4828	0.4831
16								0.4461	0.4756	0.4783	0.4947	0.5012	0.5045	0.4988	0.4904	0.2374	0.4995
17.5								0.2043						0.2237	0.2249	0.2423	0.2400
18.5				0.7497	0.7977	0.7343	0.6381	0.5236									0.7858
Total Avg																	**0.4905**

(d)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I	0.2883	0.3549	0.3948	0.4036	0.3928	0.3738											0.4296
class II			0.3959	0.4079	0.4079	0.4036	0.3957										0.4385
class III	0.2485	0.3002	0.3401	0.3459	0.3367	0.3305	0.3263	0.3222	0.3199	0.3167	0.315	0.314	0.3117	0.3112	0.3097	0.2921	0.4227
0.3		0.4643	0.4985	0.4755	0.4397	0.391											0.4997
0.7	0.4714	0.5008	0.4995	0.4818	0.457												0.5025
1	0.4065	0.4819	0.5282	0.5531	0.5628	0.5664	0.5539	0.5438	0.5349	0.523	0.4624						0.5645
1.1				0.1387				0.16	0.1643	0.1687	0.1699	0.1718	0.173	0.1739	0.1757	0.1740	0.1740
1.2				0.216	0.2513	0.2713	0.2804	0.275									0.2799
1.3		0.4186	0.5283	0.5478	0.4803	0.4071											0.5569
1.4		0.577	0.6844	0.6923	0.6164	0.5435											0.7050
1.7				0.5342	0.5443	0.5498	0.5561	0.5573	0.5559								0.5547
2				0.4689								0.4859	0.4864	0.4864	0.4881	0.4949	0.4939
2.5				0.1772				0.2	0.2022	0.2041	0.2051	0.2068	0.2056	0.2059	0.207	0.1781	0.2058
3.5				0.3184							0.3794	0.3833	0.3871	0.3896	0.3913	0.4121	0.4130
5				0.2643	0.2678	0.2679	0.267	0.2641									0.2693
6				0.3855	0.4053	0.4187	0.4234	0.4264	0.4247								0.4277
8	0.1458	0.3046	0.467	0.3768	0.2387	0.159	0.1167										0.4753
9				0.2789							0.3338	0.3388	0.3407	0.3454	0.3478	0.3875	0.3882
10A				0.3394									0.3932	0.3959	0.3984	0.4527	0.4536
11				0.29									0.3281	0.329	0.3336	0.369	0.3693
15				0.4308									0.4468	0.4487	0.4487	0.4601	0.4607
16				0.2615						0.3788	0.3759	0.3843	0.3897	0.3931	0.3962	0.3249	0.4035
17.5				0.1675								0.1945	0.1945	0.1964	0.1976	0.2079	0.2084
18.5			0.5252	0.5936	0.6135	0.5996	0.5548	0.5049									0.6224
Total Avg																	**0.4299**

(e)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I	0.4744	0.5207	0.3139	0.2033	0.1527	0.1238											0.6007
class II		0.4178	0.4294	0.4122	0.3965	0.377											0.504
class III	0.3186	0.3674	0.3616	0.3577	0.353	0.3509	0.3466	0.3455	0.3431	0.3418	0.3428	0.3404	0.3395	0.3376	0.3394	0.3087	0.5314
0.3	0.5158	0.6247	0.4561	0.302	0.2138												0.6501
0.7	0.6824	0.4816	0.3238	0.2235	0.1669												0.6983
1	0.684	0.6413	0.3624	0.2166	0.1435												0.6915
1.1		0.2044	0.2687	0.3088	0.318	0.2969	0.2693	0.2325	0.2055	0.1851							0.3287
1.2	0.2187	0.7568	0.3078	0.109	0.0593	0.0419											0.6573
1.3	0.6279	0.4296	0.157	0.0806	0.0524												0.5781
1.4	0.5258	0.7024	0.5844	0.4281	0.3137												0.705
1.7	0.5558	0.5701	0.5778	0.5744	0.5749					0.5507					0.519	0.3705	0.5798
2			0.4773								0.5017	0.5052	0.5055	0.5061	0.5061	0.5074	0.5104
2.5			0.1978	0.2192	0.2295	0.2375	0.237	0.2368	0.2357	0.237	0.2309	0.2235	0.2213			0.127	0.2332
3.5				0.3587							0.4875	0.4901	0.4974	0.4939	0.4997	0.4535	0.5
5		0.3096	0.3348	0.3379	0.3244	0.3073											0.3032
6	0.3278	0.4696	0.6226	0.6935	0.6651	0.6108	0.5367										0.6961
8	0.2634	0.3311	0.1075	0.0483													0.344
9		0.2801										0.5483	0.5495	0.5616	0.5675	0.5115	0.5755
10A		0.3351										0.5104	0.5223	0.531	0.5379	0.7012	0.7099
11		0.2901											0.4388	0.4477	0.4508	0.5693	0.5724
15		0.4328											0.4934	0.4938	0.496	0.5207	0.5206
16		0.2565	0.3346	0.4394	0.5207	0.5615	0.5755	0.5598	0.5257	0.4852	0.4565	0.4243	0.383	0.3644	0.3358	0.1039	0.5725
17.5		0.1694								0.2785		0.2891	0.2942	0.2974	0.2979	0.2631	0.2979
18.5	0.3637	0.6581	0.2353	0.2506	0.2655												0.6581
Total Avg																	**0.542**

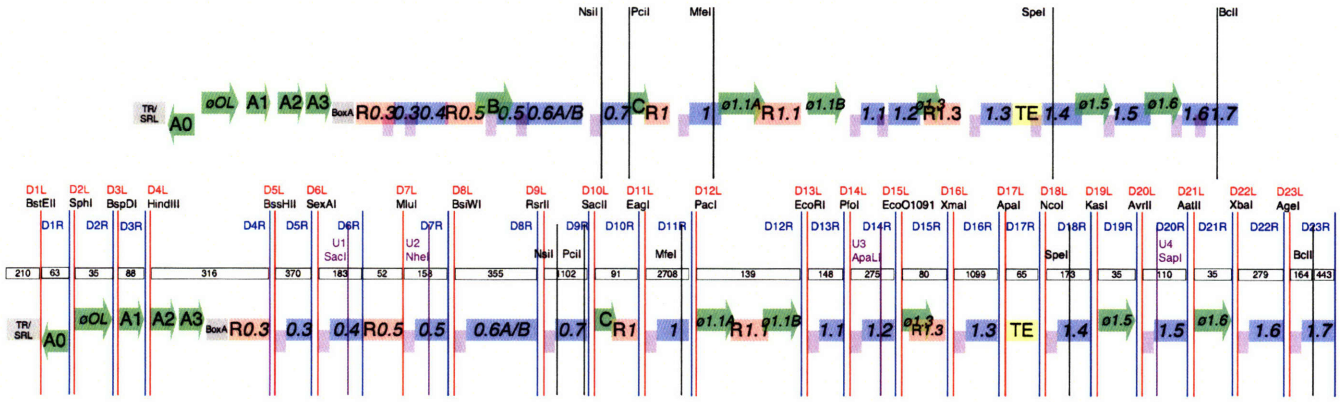
(f)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	None	Final
class I	0.3237	0.363	0.351	0.3248	0.2984	0.2745											0.3927
class II		0.3433	0.3442	0.3383	0.3308	0.327											0.3646
class III					0.287	0.2958	0.3006	0.3038	0.3082	0.3063	0.3085	0.3099	0.309	0.3071	0.3089	0.2881	0.3159
0.3	0.3767	0.4121	0.4056	0.374	0.3468												0.4112
0.7	0.472	0.4513	0.414	0.3785	0.3464												0.4737
1	0.5441	0.5955	0.5644	0.5145	0.4575												0.5972
1.1		0.1222		0.1348		0.1446	0.1484	0.1515	0.1518	0.154	0.155	0.1556	0.1575	0.1585	0.1587	0.1655	0.1663
1.2		0.1364		0.199		0.231	0.2363	0.2402	0.2348	0.2278		0.22		0.217			0.2361
1.3	0.3578	0.4701	0.4477	0.3782	0.3048	0.2492											0.4719
1.4	0.4977	0.6292	0.6129	0.503	0.4397												0.6387
1.7			0.4634	0.4676	0.4714	0.4737	0.4751	0.4766	0.4777	0.478	0.4782	0.4792	0.4796	0.4801	0.4802	0.4794	0.4772
2			0.4468								0.4524	0.4524	0.4528	0.453	0.4534	0.4553	0.4542
2.5			0.1361						0.1428	0.1435	0.1439	0.1442	0.1443	0.1445	0.1449	0.1477	0.1465
3.5			0.2575								0.2728	0.2747	0.2747	0.2756	0.2755	0.2849	0.283
5			0.2215		0.2246	0.2258	0.2259	0.2271	0.2271	0.2275	0.2272	0.2276	0.2275	0.2274	0.2272	0.2234	0.2274
6			0.2781							0.3104	0.3125	0.316	0.3162	0.3165	0.3174	0.3263	0.3255
8					0.1972	0.219	0.2279	0.2356	0.2579	0.2431	0.2431	0.262	0.26	0.265	0.2609	0.1967	0.2601
9												0.2485	0.2484	0.2499	0.2511	0.2603	0.2611
10A												0.3138	0.3145	0.3147	0.3152	0.3238	0.325
11												0.2679	0.2683	0.269	0.269	0.2756	0.276
15										0.4329	0.4326	0.4332	0.4345	0.4347	0.435	0.442	0.4413
16												0.2882	0.2823	0.2879	0.2897	0.327	0.3363
17.5												0.1575	0.1583	0.1571	0.158	0.1648	0.1666
18.5		0.2819	0.3181	0.3489	0.3812	0.3972	0.4049	0.415	0.4357	0.4373	0.4405	0.4474	0.461	0.4533	0.4511	0.4238	0.461
Total Avg																	**0.3541**

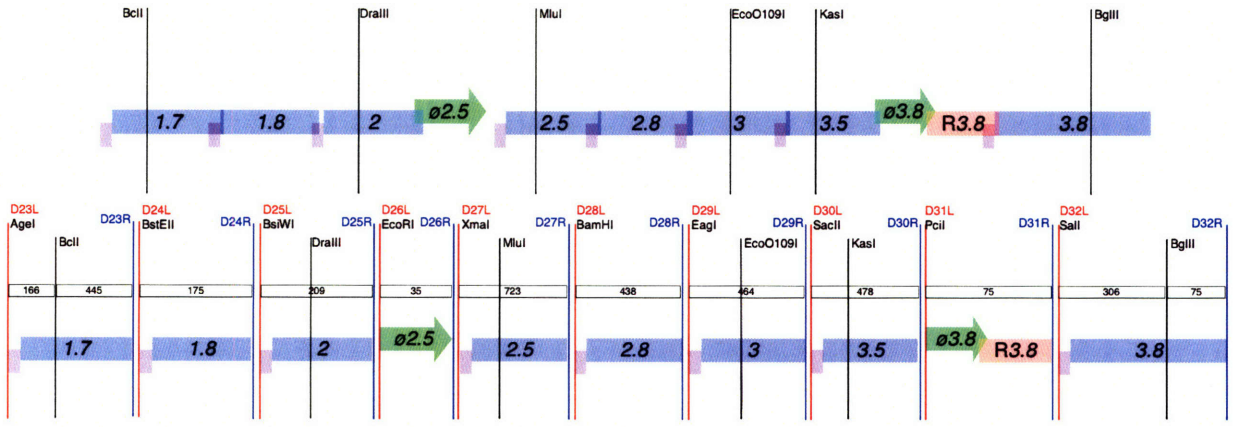
Chapter 7.2. Refactoring Supplemental Information

Figure 7-3. Genome design. We split the wild-type T7 genome into six sections, alpha through zêta, using five restriction sites unique across the natural sequence. Each section shown here has a wild-type section with representations of the genetic elements: protein coding regions (blue), ribosome binding sites (purple), promoters (green), RNase III recognition sites (pink), transcription terminators (yellow), and others (gray). Elements are labeled by convention [7]. Images are not to scale, but overlapping boundaries indicate elements with shared sequence. The useful natural restriction sites across each section are shown (black lines). T7.1 sections are shown below the wild-type sections. Parts are given integer numbers, 1 through 73, starting at the left end of the genome. Unique restriction site pairs bracket each part (red/blue lines, labeled D[part #]L/R). Added unique restriction sites (purple lines, U[part #]) and part length (# base pairs, open boxes) are shown.

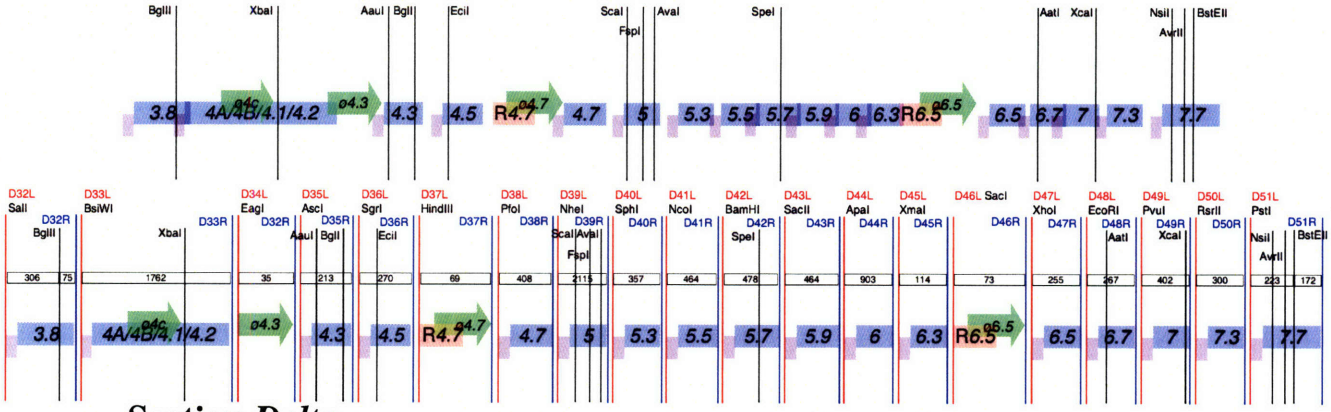
Section *Alpha*



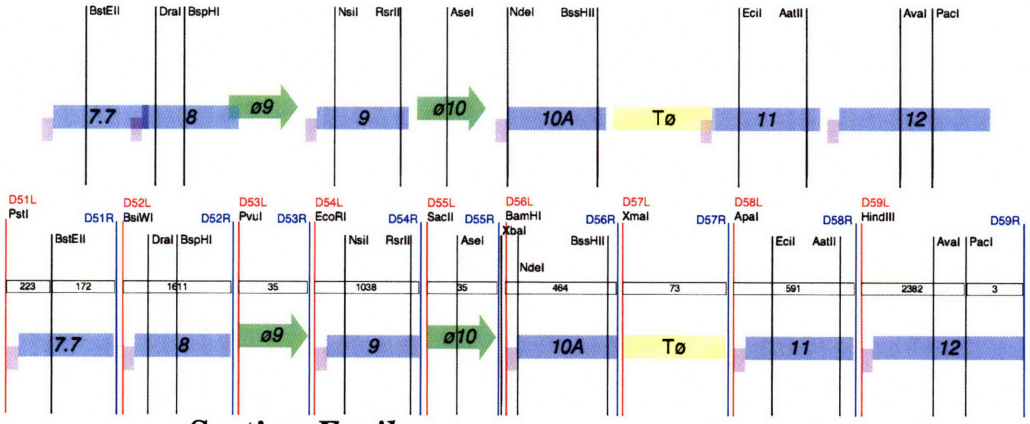
Section *Beta*



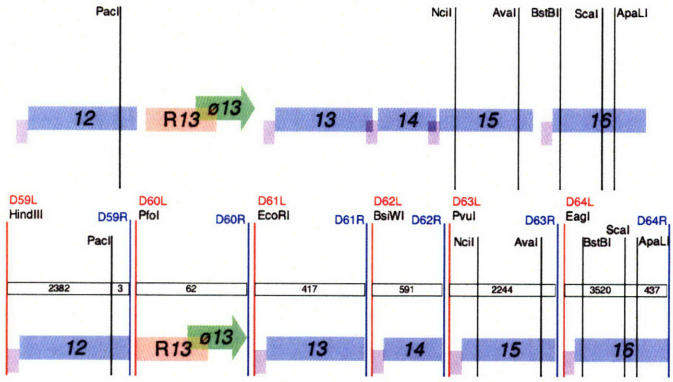
Section *Gamma*



Section *Delta*



Section *Epsilon*



Section *Zêta*

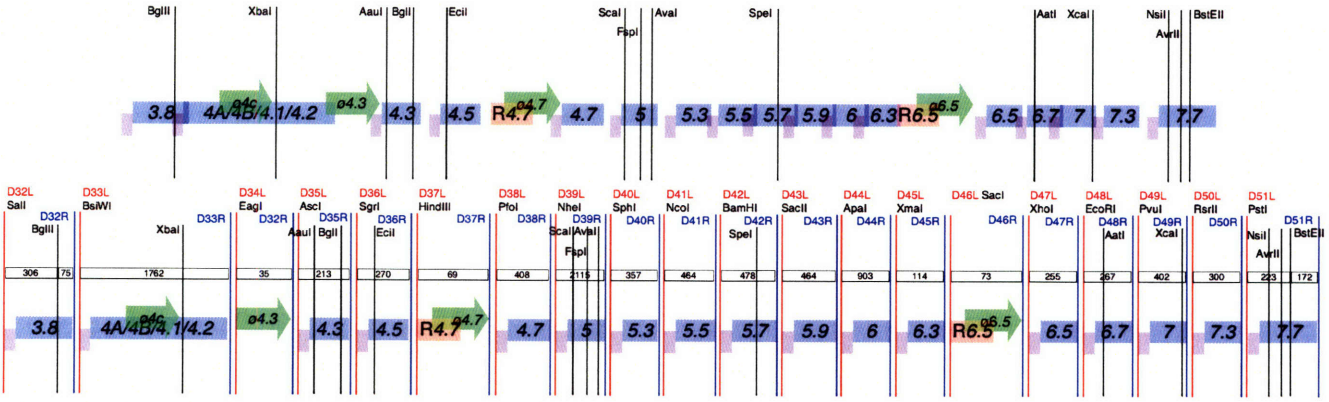


Figure 7-4. Differences between wild-type T7 and T7.1. A listing from left to right on the T7 genome of the changes made during design of T7.1. Changes are shown by comparison of the annotated wild-type T7 (above) and T7.1 (below) sequences. Point mutations are capitalized. The natural ribosome binding sites are underlined. The bracketing restriction sites surrounding parts are orange (left cutter) and blue (right cutter), with overlaps of neighboring bracketing restriction sites in light green. Other features include start codons (green), stop codons (red), and overlaps of start and stop codons (purple).

Changes in Section *Alpha*

```
LeftEnd-Part1: TR-SRL/A0
T7:       gagtgtctctctgtgtccctatctgttacagtctcctaaagtatcctcct
          ------TR---------->             <-------SRL-----
T7.1:     gagtgtctctctgtgtccctatcGgttacCgtctcctaaagtatcctcct
          ------TR---------->    <-D1L->    <-------SRL-----


Part1-Part2: SRL/A0-øOL
T7:       acctaaagacgccttgttgttagccataaagtgataacctttaatcattgtctttattaa
          --SRL-->                                    <-----øOL----
T7.1:     acctaaagGTTaccgcatgcttgttgttagccataaagtgataacctttaatcattgtctttattaa
          --SRL-->      <D2L->                                <-----øOL----
                <-D1R->


Part2-Part3: øOL-A1
T7:       aaggagagacaacttaaagagacttaaaagattaatttaaaatttatcaaaaag
          ---ø0L-->                                 <---A1---
T7.1:     aaggagagacaacttaaagagCAtgcttaaaagattaatcgattaaaatttatcaaaaag
          ---øOL-->           <D2R->           <D3L->        <---A1---


Part3-Part4: A1-A2/A3/BoxA/R0.3
T7:       gagagggacacggcgaatagccatcccaatcgacaccggggtcaaccggataagtagacagcctgataagtcgcacgaaaaacagg
          ----A1----->                                                          <----A2----
T7.1:     gagagggacacggcgaatagccatcccaatcgaTaccggggtcaaccggataagtagaAagcTtgataagtcgcacgaaaaacagg
          ----A1----->                <D3R->                          <D4L->          <----A2----


Part4-Part5: A2/A3/BoxA/R0.3-0.3
T7:       gatattcactaataactgcacgaggtaacacaagatggctatgtctaaca
          ---R0.3--->        <-0.3RBS->     <------0.3------
T7.1:     gatattcactaagcttgcgcgctgcacgaggtaacacaagatggctatgtctaaca
          ---R0.3--->     <D5L->   <-0.3RBS->     <------0.3------
                    <D4R->


Part5-Part6: 0.3-0.4
T7:       cgaggagtacgaggaggatgaagagtaatgtctactacc
          -------------0.3---------->
                  <-0.4RBS->        <----0.4----
T7.1:     cgaggagtacgaggaggaCgaagagtaagcgcgccaccaggtcgaggagtacgaggaggatgaagagtaatgtctactacc
          -------------0.3----------><D5R-><-D6L->         <-0.4RBS->        <----0.4----


U1-R0.5: U1/0.4-R0.5
T7:       caaagaactgtacgaaaacaacaaggcaatagctttagaatctgctgagtgatagactcaaggtc
          ----------------------------------------------0.4---->
                                                        <-------R0.5-------
T7.1:     caaagaGctCtacgaaaacaacaaggcaatagctttagaatctgctgagtgaaccaggtgagtgatagactcaaggtc
          ----------------------------------------------0.4----><-D6R-><-------R0.5-------
              <-U1->


R0.5-Part7: R0.5-0.5
T7:       gcctttatgattatcactttacttatgagggagtaatgtatatgctt
          -------R0.5------->      <-0.5RBS-><----0.5----
T7.1:     gcctttatgattatcacttacgcgtcttatgagggagtaatgtatatgctt
          -------R0.5-------><D7L->    <-0.5RBS-><----0.5----


U2
T7:       gctctaggtctagctgtaggtgcatcc
          ---------------0.5---------
T7.1:     gctctaggGctagctgtaggtgcatcc
          ---------------0.5---------
                    <-U2->
```

Part7-Part8: 0.5-0.6A/B

```
T7:       catcaaaggggcactacgcaaatgatgaagcac
          ---------0.5------------>
             <-0.6RBS->         <----0.6----
T7.1:     catcaaaggCgcactacgcaaatAaacgcgtacgcaaaggggcactacgcaaatgatgaagcac
          ---------0.5------------><D7R->   <-0.6RBS->        <----0.6----
                                    <D8L->
```

Part8-Part9: 0.6A/B-0.7

```
T7:
          aacaggcactagccaacacactgaacgctatctcataacgaacataaaggacacaatgcaatgaacattacc
          ----0.6----->                                <-0.7RBS->      <----0.7----
T7.1:
          aacaggcactagcgtacggtccgcgaacataaaggacacaatgcaatgaacattacc
          ----0.6----><D8R->           <-0.7RBS->      <----0.7----
                      <-D9L->
```

Part9-Part10: *0.7*-C/R*1*

```
T7:       caacattgataagcaacttgacgcaatgttaatgggctgatagtcttatct
          --------------------0.7-----------------> <----R1---
              <-------------------------C----------------------
T7.1:     caacattgataagcaacttgacgcaatgttaatgggctgacggtccgccgcggattgataagcaacttgacgcaatgttaatgggctgatagtcttatct
          --------------------0.7-----------------><-D9R-><D10L><------------------------C----------------------
                                                                                                  <----R1---
```

Part10-Part11: C/R*1-1*

```
T7:       ataggtacgatttactaactggaagaggcactaaatgaacacgatt
          ----R1------>          <-1RBS->    <-----1-----
T7.1:     ataggtacgatttactaacccgcggccgctggaagaggcactaaatgaacacgatt
          ----R1----->       <D10R>       <-1RBS->    <-----1-----
                             <D11L>
```

Part11-Part12: *1*-ø*1.1*A/R*1.1*/ø*1.1*B

```
T7:       gcgttcgcgtaacgccaaatcaatacgactcactatagagggacaaac
          -----1----->                        <---R1.1----
                    <----------------ø1.1A----------------->
T7.1:     gcgttcgcgtaacggccgttaattaaaacgccaaatcaatacgactcactatagagggacaaac
          -----1-----><D11R><-D12L-><----------------ø1.1A----------------->
                                                    <---R1.1----
```

Part12-Part13: ø*1.1*A/R*1.1*/ø*1.1*B-*1.1*

```
T7:       tataggagaaccttaaggtttaactttaagacccttaagtgttaattagagatttaaattaaagaattactaagaggaggactttaagtatgcgtaacttc
          ---ø1.1B--->                                                                  <-1.1RBS->      <----1.1----
T7.1:     tataggagaaccttaaggtttaactttaagacccttaagtgttaattaAagatttaaattaaagaattCctaagaggaggactttaagtatgcgtaacttc
          ---ø1.1B--->                              <-D12R->            <D13L>   <-1.1RBS->      <----1.1----
```

Part13-Part14: *1.1-1.2*

```
T7:       ctgggagggtcagtaagatgggacgttta
          ------1.1------> <----1.2----
          <-1.2RBS->
T7.1:     ctgggagggtcagtaagaattccaggactgggagggtcagtaagatgggacgttta
          ------1.1------><D13R>     <-1.2RBS->       <----1.2----
                          <D14L->
```

U3

```
T7:       gacgaggacgttctgttcaatatgtgtactgattggttgaaccat
          ----------1.2-----------------------------------
T7.1:     gacgaggacgttctgttcaatatgtgCactgattggttgaaccat
          ----------1.2-----------------------------------
                               <-U3->
```

Part14-Part15: *1.2*-ø*1.3*/R*1.3*

```
T7:       gttgaaggactggaagtaatacgactcagtatagggacaa
          --------1.2-------->          <---R1.3---
                <--------------ø1.3---------------
T7.1:     gttgaaggactggaagtaatccaggacccggactggaagtaatacgactcagtatagggacaa
          --------1.2-------><D14R->   <--------------ø1.3----------------
                              <D15L->                       <---R1.3---
```

Part15-Part16: ø*1.3*/R*1.3*-*1.3*

```
T7:       atttaaccaataggagataaacattatgatgaacatt
          ----R1.3--->             <----1.3----
                   <-1.3RBS->
T7.1:     atttaaccaataggaggacccgggccaataggagataaacattatgatgaacatt
          ----R1.3--->      <D16L>   <-1.3RBS->      <----1.3----
                      <D15L->
```

Part16-Part17: *1.3*-TE

```
T7:       agagaaaatgtaatcacactggctcaccttcgggtgggcctt
          -----1.3---->    <----------TE------------
T7.1:     agagaaaatgtaacccgggcccaaaatgtaatcacactggctcaccttcgggtgggcctt
```

```
          -----1.3---->&lt;D16R&gt;                &lt;----------TE------------
                        &lt;D17L&gt;
```

Part17-Part18: TE-*1.4*

```
T7:       gcctttctgcgtttataaggagacactttatgtttaagaag
          -TE-->        <1.4RBS >      <----1.4----
T7.1:     gcctttcagggaaaacgggccatggttataaggagacactttatgtttaagaag
          -TE-->          <D17R>       <1.4RBS >      <----1.4----
                            <D18L>
```

Part18-Part19: *1.4-ø1.5*

```
T7:       cgtgtggagtatagttaactggtaatacgactcactaaagg
          -----------1.4---------->
                    <---------ø1.5--------------
T7.1:     cgtgtggagtatagttaactggtaatccatggcgccgttaactggtaatacgactcactaaagg
          -----------1.4----------> <D18R>    <---------ø1.5--------------
                                       <D19L>
```

Part19-Part20: *ø1.5-1.5*

```
T7:       taaaggaggtacacaccatgatgtactta
          ----ø1.5--->     <----1.5----
           <-1.5RBS->
T7.1:     taaaggaggtacggcgcctaggcactaaaggaggtacacaccatgatgtactta
          ----ø1.5--->&lt;D19R&gt;       <-1.5RBS->      <----1.5----
                        <D20L>
```

U4

```
T7:       gtcattgtaggatgccttgcgctccactgtagcgatgat
          -------1.5-------------------------------
T7.1      gtcattgtaggatgccttgcTctTcactgtagcgatgat
          -------1.5-------------------------------
                          <-U4-->
```

Part20-Part21: *1.5-ø1.6*

```
T7:       tgccagatggtcacgcttaatacgactcact
          --------1.5-------->
                  <--------ø1.6-----------
T7.1:     tgccagatggtcacgcttaacctaggacgtctggtcacgcttaatacgactcact
          --------1.5--------><D20L>     <--------ø1.6-----------
                                <D21L>
```

Part21-Part22: *ø1.6-1.6*

```
T7:       taaaggagacactatatgtttcgactt
          ----ø1.6--->   <----1.6----
          <-1.6RBS->
T7.1:     taaaggagacacgacgtctagacactaaaggagacactatatgtttcgactt
          ----ø1.6--->&lt;D21R&gt;       <-1.6RBS->     <----1.6----
                        <D22L>
```

Par22-Part23: *1.6-1.7*

```
T7:       tcaaggaggtgttctgatgggactgtta
          -------1.6------>
           <--1.7RBS-->   <----1.7----
T7.1:     tcaaAgaAgtgttctgatctagaaccggttcaaggaggtgttctgatgggactgtta
          -------1.6------><D22R><D23L> <--1.7RBS-->   <----1.7----
```

Changes in Section *Beta*

Part23-Part24: *1.7-1.8*

```
T7:       gaactctttgagaaacataaggataaatgttatgcataacttcaagtca
          -----------------1.7------------------>
                        <-1.8RBS->      <------1.8--------
T7.1:     gaactctttgagaaacataaAgataaatgttaCgcataaccggtgacctctttgagaaacataaggataaatgttatgcataacttcaagtca
          -----------------1.7------------------>  <D24L->          <-1.8RBS->      <-----1.8---------
                                                 <D23R>
```

Part24-Part25: *1.8-2*

```
T7:       gaactttggaaatcgagaggtcaatgactatgtcaaacgta
          ------------1.8----------->  <-----2-----
                        <--RBS-->
T7.1:     gaactttggaaatcgagaggtcaatgaggtcaccgtacgttggaaatcgagaggtcaatgactatgtcaaacgta
          ------------1.8----------><D24R>               <--2RBS-->     <-----2-----
                                       <D25L>
```

Part25-Part26: *2-ø2.5*

```
T7:       ttgtgtagcaccgaagtaatacgactcactat
          ---------2-------->
                <-----------ø2.5----------
T7.1:     ttgtgtagcaccgaagtaacgtacgaattcagcaccgaagtaatacgactcactat
          ---------2--------><D25R>     <------------ø2.5---------
                               <D26L>
```

Part26-Part27: *ø2.5-2.5*

```
T7:       cactattagggaagactccctctgagaaaccaaacgaaacctaaaggagattaacattatggctaagaag
          -----ø2.5----->                          <-2.5RBS->       <----2.5----
T7.1:     cactattagggaagagaattcccgggcgaaacctaaaggagattaacattatggctaagaag
          -----ø2.5-----><D26R>           <-2.5RBS->       <----2.5----
                              <D27L>
```

Part27-Part28: *2.5-2.8*

```
T7:       gcagacgaagacggagacttctaagtggaactgcgg
          ---------2.5----------><----2.8----
                    <-2.8RBS->
T7.1:     gcagacgaagacggGgacttctaacccggatccgaagacggagacttctaagtggaactgcgg
          ---------2.5----------><D27R>       <-2.8RBS->     <----2.8----
                                    <D28L>
```

Part28-Part29: *2.8-3*

```
T7:       acgcaaagggaggcgacatggcaggttacggcgctaaaggaatccgaaa
          ----------------2.8----------------->
              <--3RBS-->   <-----------------3---------------
T7.1:     acgcaaGgggagAcgacaCggcaggttacggcgctaaggatccggccgcaaagggaggcgacatggcaggttacggcgctaaaggaatccgaaa
          ----------------2.8-----------------><D28R>      <--3RBS-->   <---------------3---------------
                                                 <D29L>
```

Part29-Part30: *3-3.5*

```
T7:       gattaaaaaggaaaggaggaaagaaataatggctcgtgta
          ------------3--------------->
                    <-3.5RBS->        <----3.5----
T7.1:     gattaaaaCgCaaGggGggGaagaaataacggccgccgcggaaaggaaaggaggaaagaaataatggctcgtgta
          ------------3---------------><D29R><D30L>    <-3.5RBS->        <----3.5----
```

Part30-Part31: *3.5-ø3.8*/R*3.8*

```
T7:       tctgaccgtggataattaattgaactcactaaag
          ------3.5----->
                <------------ø3.8-----------
T7.1:     tctgaccgtggataaccgcggacatgtcgtggataattaattgaactcactaaag
          ------3.5-----><D30R><D31R><------------ø3.8-----------
```

Part31-Part32: *ø3.8*/R*3.8-3.8*

```
T7:       tttccctttgttcgcattggaggtcaaataatgcgcaagtct
          -------R3.8------>            <----3.8----
                         <-3.8RBS->
T7.1:     tttccctttgttcgcattggaggtcaaataatacatgtcgacgaggtcaaataatgcgcaagtct
          -------R3.8------>              <D31R>  <-3.8RBS->   <----3.8----
                                             <D32L>
```

Changes in Section *Gamma*

Part32-Part33: *3.8-4A/4B/4.1/4.2*

```
T7:       tagaactaggagggaattgcatggacaattcgcacgattccgatagtgt
          ---------------------3.8----------------------->
                <-4ARBS->     <-------------4A-------------
T7.1:     tagaactagAagAgaattgcaCggacaattcgcacgattccgatagtgtcgacgtacgctaggagggaattgcatggacaattcgcacgattccgatagtgt
          ---------------------3.8-----------------------><D32R>      <-4ARBS->     <------------4A--------------
                                                           <D33L>
```

Part33-Part34: *4A/4B/4.1/4.2-ø4.3*

```
T7:       ggagagtcccattctaatacgactcactaaa
          -------4.2------>
              <------------ø4.3----------
T7.1:     ggagagtcccattctaacgtacggccgagtcccattctaatacgactcactaaa
          -------4.2------><D33R>    <------------ø4.3----------
                              <D34L>
```

Part34-Part35: *ø4.3-4.3*

```
T7:       ctaaaggagacacaccatgttcaaactg
          ----ø4.3---->   <----4.3----
           <-4.3RBS->
T7.1:     ctaaaggagacacaccggccggtggcgcgcctaaaggagacacaccatgttcaaactg
          ----ø4.3---->  <D34R>   <D35L> <-4.3RBS->     <----4.3----
```

Part35-Part36: *4.3-4.5*

```
T7:       ttctttgagtaatcaaacaggagaaaccattatgtctaacgta
          ----4.3---->    <-4.5RBS->     <----4.5----
T7.1:     ttctttgagtaatggcgcgccaccggcgaaacaggagaaaccattatgtctaacgta
          ----4.3---->  <D35R><-D36L->  <-4.5RBS->     <----4.5----
```

Part36-Part37: *4.5-R4.7/ø4.7*

```
T7:       attgataactaagagtggtatcct
          ----4.5----><----R4.7---
T7.1:     attgataactaacaccggcgaagcttaagagtggtatcct
          ----4.5----><-D36R-><D37L> <----R4.7---
```

Part37-Part38: *R4.7/ø4.7-4.7*

```
T7:       ctataggagatattaccatgcgtgaccct
```

```
          ----ø4.7---->    <----4.7----
            <-4.7RBS->
T7.1:     ctataggagatattaccaagcttcctggactataggagatattaccatgcgtgaccct
          ----ø4.7---->    <D37R>        <-4.7RBS->     <----4.7----
                               <D38L->
```

Part38-Part39: *4.7-5*

```
T7:       aagtcacgataatcaataggagaaatcaatatgatcgtttct
          ----4.7---->   <-5RBS->       <-----5-----
T7.1:     aagtcacgataatcctggagctagcatcaataggagaaatcaatatgatcgtttct
          ----4.7---->D38R-><D39L>    <-5RBS->       <-----5-----
```

Part39-Part40: *5-5.3*

```
T7:       atttgccactgatacaggaggctactcatgaacgaaaga
          -----5-----> <-5.3RBS->    <----5.3----
T7.1:     atttgccactgagctagcatgctacaggaggctactcatgaacgaaaga
          -----5----->D39R>     <-5.3RBS->    <----5.3----
                          <D40L>
```

Part40-Part41: *5.3-5.5*

```
T7:       ataaaactataggagaaattattatggctatgaca
          ----5.3---->           <----5.5----
                 <-5.5RBS->
T7.1:     ataaaactatagcatgccatggtataggagaaattattatggctatgaca
          ----5.3---->    <D41L> <-5.5RBS->     <----5.5----
                    <D40R>
```

Part41-Part42: *5.5-5.7*

```
T7:       acgggaggtgttctgatgtctgactac
          ------5.5------>
           <-5.7RBS->    <----5.7----
T7.1:     acgCgaggtgttctgaccatggatccgggaggtgttctgatgtctgactac
          ------5.5------><41R>    <-5.7RBS->    <----5.7----
                             <42L>
```

Part42-Part43: *5.7-5.9*

```
T7:       tgggaggatgtgtctaatgtctcgtgac
          -------5.7------>
          <-5.9RBS->      <----5.9----
T7.1:     tgggCggGtgtgtctaaggatccgcggcgaatgggaggatgtgtctatgtctcgtgac
          -------5.7------><D42R>        <-5.9RBS->     <----5.9----
                               <D43L>
```

Part43-Part44: *5.9-6*

```
T7:       ctagaggagaaacttaatggcacttctgacc
          ----------------5.9----------->
          <-6RBS->        <--------6------
T7.1:     ctagaAgaAaaacttaaCggcacttctgaccgcgggcccggaactagaggagaaacttaatggcacttcttgacc
          ----------------5.9----------><D43R>        <-6RBS->        <--------6------
                                          <D44L>
```

Part44-Part45: *6-6.3*

```
T7:       gacaaggagatttacctgtggagaccgtagcgt
          --------------6-------------->
            <-6.3RBS->     <------6.3------
T7.1:     gacaaggaAatttacctCtggagaccgtaggcccgggcaaggagatttacctgtggagaccgtagcgt
          --------------6--------------> <D45L><-6.3-RBS->    <------6.3------
                                    <D44R>
```

Part45-Part46: *6.3-R6.5/ø6.5*

```
T7:       gacactaagtgataaact
          ----6.3---->
                <---R6.5----
T7.1:     gacactaaAtAacccggagctcactaagtgataaact
          ----6.3----><D45R>        <---R6.5----
                          <D46L>
```

Part46-Part47: *R6.5/ø6.5-6.5*

```
T7:       cgattattactttaagatttaactctaagaggaatctttattatgttaacacct
          ----R6.5---->            <-6.5RBS->       <----6.5----
T7.1:     cgattattactttaagatttaagagctcgagtaagaggaatctttattatgttaacacct
          ----R6.5---->         <D46R>   <-6.5RBS->       <----6.5----
                                   <D47L>
```

Part47-Part48: *6.5-6.7*

```
T7:       tgatggggaggattgacactatgtgtttctca
          ------6.5------>    <----6.7----
            <-6.7RBS->
T7.1:     tgatggCgaAgattgactcgagaattctgatggggaggattgacactatgtgtttctca
          ------6.5------><D47R>       <-6.7RBS->        <----6.7----
                             <D48L>
```

Part48-Part49: *6.7-7*

```
T7:       tttggaggtaagaagtgatgtctgagttc
```

```
          ------6.7-------->
            <-7RBS->       <-----7-----
T7.1:     tttggaggtaagaagtgagaattcgatcgcatttggaggtaagaagtgatgtctgagttc
          ------6.7-------><D48R>         <-7RBS->       <-----7-----
                                <D49L>
```

Part49-Part50: *7-7.3*

```
T7:       tttaaggaggtataagttatgggtaagaaa
          -------7------>   <----7.3----
           <-7.3RBS->
T7.1:     tttaaggaggtataacgatcggtccgctttaaggaggtataagttatgggtaagaaa
          -------7------><D49L>       <-7.3RBS->       <----7.3----
                            <D50R->
```

Part50-Part51: *7.3-7.7*

```
T7:       atcaacatttaatcaggaggttatcgtggaagactgc
          ----7.3----> <-7.7RBS->  <----7.7----
T7.1:     atcaacatttaacggtccgctgcagtcaggaggttatcgtggaagactgc
          ----7.3----><D50R-><D51L> <-7.7RBS->  <----7.7----
```

Changes in Section *Delta*

Part51-Part52: *7.7-8*

```
T7:       gacatggagacacatttaatggctgagaaa
          -------7.7-------->
             <-8RBS->       <-----8-----
T7.1:     gacatggagacacatttaactgcagcgtacgagacatggagacacatttaatggctgagaaa
          -------7.7-------><D51R><D52L>    <-8RBS->       <-----8-----
```

Part52-Part53: *8-ø9*

```
T7:       cagccgggaatttaatacgactcactatag
          -------8------>
            <------------ø9-------------
T7.1:     cagccgggaatttaacgtacgatcgccgggaatttaatacgactcactatag
          -------8------><D52L>   <------------ø9-------------
                            <D53L>
```

Part53-Part54: *ø9-9*

```
T7:       tagggagacctcatctttgaaatgagcgatgacaagaggttggagtcctcggtcttcctgtagttcaacttaaggagacaataataatggctgaat
          ----ø9--->                                                                  <-9RBS->         <----9----
T7.1:     tagggagacctcatctttgaaatgagcgatcgacaagaggttggagtcctcggtcttcctgtagaattcaacttaaggagacaataataatggctgaat
          ----ø9--->               <D53R>                                  <D54L>    <-9RBS->         <----9----
```

Part54-Part55: *9-ø10*

```
T7:       tcgaacttctgatagacttcgaaatta
          -----9----->   <----ø10----
T7.1:     tcgaacttctgatagaattccgcggacttcgaaatta
          -----9-----> <D54R>     <----ø10----
                            <D55L>
```

Part55-Part56: *ø10-10A/B*

```
T7:       tatagggagaccacaacggtttccctctagaaataattttgtttaactttaagaaggagatatacatatggctagcatg
          ----ø10---->                                        <-10RBS->       <----10-----
T7.1:     tatagggagaccacaaccgcggatcctctagaaataattttgtttaactttaagaaggagatatacatatggctagcatg
          ----ø10---->    <D55R>                              <-10RBS->       <----10-----
                             <D56L>
```

Part56-Part57: *10A/B-Tø*

```
T7:       gctgagcaataactagcataaccccttggggcct
          ----10----->     <-------Tø-------
T7.1:     gctgagcaataaggatcccgggctagcataaccccttggggcct
          ----10-----><D56R>         <-------Tø-------
                          <D57L>
```

Part57-Part58: *Tø-11*

```
T7:       gttttttgctgaaaggaggaactatatgcgctcata
          --Tø-->   <-11RBS->      <----11----
T7.1:     gttttttgctgacccgggcccctgaaaggaggaactatatgcgctcata
          --Tø-->     <D57R>    <-11RBS->      <----11----
                        <D58L>
```

Part58-Part59: *11-12*

```
T7:       tgactcgctaacattaataaataaaggaggctctaatggcactcat
          ----11----->         <-12RBS->     <----12----
T7.1      tgactcgctaagggcccaagcttaataaataaaggaggctctaatggcactcat
          ----11----><D58R><D59L>     <-12RBS->     <----12----
```

Changes in Section *Epsilon*

Part59-Part60: *12-R13/ø13*

```
T7:       ccggtatttaataaatattctccctgtgg
          ----12----->      <----R13----
```

```
T7.1:     ccggtatttaattaaagcttcccggattctccctgtgg
          ----12---->   <D59L>      <----R13----
                           <D60R->
```

Part60-Part61: R*13/ø13-13*

```
T7:       tatagggagaacaatacgactacgggagggttttcttatgatgactat
          ----ø13---->          <-13RBS->      <----13----
          ----R13--->
T7.1:     tatagggagaacaatactcccggaattctacgggagggttttcttatgatgactat
          ----ø13---->     <D60R->      <-13RBS->      <----13----
          ----R13--->           <D61L>
```

Part61-Part62: *13-14*

```
T7:       acgaaaggaggataaccatatgtgttgggc
          ------13------>    <----14----
             <-14RBS->
T7.1:     acgaaaggCggCtaagaattcgtacgcacgaaaggaggataaccatatgtgttgggc
          ------13------><D61R>          <-14RBS->       <----14----
                            <D62L>
```

Part62-Part63: *14-15*

```
T7:       gacggggaggtaatgagctatgagtaaaat
          -----14----->      <----15----
            <-15RBS->
T7.1:     gacCggCaggtaacgtacgatcgccaagacggggaggtaatgagctatgagtaaaat
          -----14-----><D62R>          <-15RBS->        <----15----
                          <D63L>
```

Part63-Part64: *15-16*

```
T7:       gtaaggagtaactaaaggctacataaggaggccctaaatggataagta
          ----15---->           <-16RBS->      <----16----
T7.1:     gtaaggagtaacgatcggccgtaaaggctacataaggaggccctaaatggataagta
          ----15----><D63R>              <-16RBS->      <----16----
                        <D64L>
```

Changes in Section *Zêta*

Part64-Part65: *16-ø17*

```
T7:       gggagcgtaggaaataatacgactcactatag
          -------16------->
              <--------------ø17----------
T7.1:     gggagcgtaggaaataacggccgcggcgtaggaaataatacgactcactatag
          -------16-------><D64R>  <--------------ø17----------
                            <D65L>
```

Part65-Part66: *ø17-17*

```
T7:       gggagaggcgaaataatcttctccctgtagtctcttagatttacttaaggaggtcaaatggctaacgt
          --ø17-->                                    <-17RBS->   <----17----
T7.1:     gggagaggcgaaataatcttctcccgcggtgtaagcttcttagatttacttaaggaggtcaaatggctaacgt
          --ø17-->              <D65R>   <D66L>             <-17RBS->   <----17----
```

Part66-Part67: *17-17.5*

```
T7:       aacgagtaattggtaaatcacaaggaaagacgtgtagtccacggatggactctcaaggaggtacaaggtgctatca
          ---17--->                                          <-17.5RBS->   <--17.5--
T7.1:     aacgagtaattggtaaatcacaagcttgaaagacgtgtagtccacggatccggactctcaaggaggtacaaggtgctatca
          ---17--->            <D66R>                 <D67L>       <-17.4RBS->   <--17.5--
```

Part67-Part68: *17.5-18*

```
T7:       caataaggagtgatatgtatggaaaagga
          ----17.5---->     <----18----
            <-18RBS->
T7.1:     caataaAgaAtgaggatcccgggaataaggagtgatatgtatggaaaagga
          ----17.5----><D67R>     <-18RBS->       <----18----
                          <D68L>
```

Part68-Part69: *18-R18.5*

```
T7:       cattacagtgatatactcaa
          ----18---->
              <-----R18.5-----
T7.1:     cattacaatagcccgggcccacattacagtgatatactcaa
          ----18----><D68R>        <-----R18.5-----
                        <D69L>
```

Part69-Part70: R*18.5-E/18.5/18.7*

```
T7:       gtcattgtctatacgagatgctcctacgtgaaatctgaaagttaacgggaggcattatgctagaatt
          -R18.5->                <--------------------E------------------
                                                  <-18.5RBS-> <---18.5---
T7.1:     gtcattgtctatacgagatgctgggcccctacgtgaattctgaaagttaacgggaggcattatgctagaatt
          -R18.5->              <D69R>     <D70L>          <-18.5RBS-> <---18.5---
```

Part70-Part71: E/*18.5/18.7-19/19.2/19.3*

```
T7:       aacgtaagtaggaaatcaagtaaggaggcaatgtgtctactca
          ---18.5---->        <-19RBS->     <----19----
T7.1:     aacgtaagtaggaattcgtacgaagtaaggaggcaatgtgtctactca
```

```
         ---18.5--->D70R>       <-19RBS->    <----19----
                        <D71L>
```

Part71-Part72: *19/19.2/19.3-øOR*

```
T7:      ggtgatttatgcattaggactgcatagggatgcactatagaccacggatggtcagttctttaagttactgaaaagacacgat
         -19->                                                                    <-øOR-
T7.1:    ggtgatttatgcattaggactgcatagggatgcactatagaccacgtacgatggtcagttctttaagttactgcagaaaagacacgat
         -19->                                  <D71R>                  <D72L>      <-øOR-
```

Part72-Part73: *øOR-19.5*

```
T7:      gagagga...94nt... gattatattgtattagtatcaccttaacttaaggaccaacataaagggaggagactcatgttcc
         -øOR->                                                  <-19.5RBS->    <-19.5-
T7.1:    gagagga...94nt... gattatattgtattagtatcaccttaactgcagtcgaccaacataaagggaggagactcatgttcc
         -øOR->                                  <D72R>          <-19.5RBS->    <-19.5-
                                                      <D73L>
```

Part73-SRR/TR: *19.5*-SRR/TR

```
T7:      cgattaggtcttcctgaccgactgatggctcaccgagggattcagcggtatgattgcatcacaccacttcatccctata
         -19.5->                                                                  <-SRR-
T7.1:    cgattaggtcgacttcctgaccgactgatggctcaccgagggattcagcggtatgattgcatcacaccacttcatccctata
         -19.5-> <D73R>                                                           <-SRR-
```

Table 7-1. Errors in synthesis & construction of section *alpha*.

Location on T7.1 (Genome Position)	Nature of Difference	Probable Reason for Difference	Expected Outcome
D1L (164-170), D1R & D2L (338-350)	Restriction sites were not added in construction	Difficulties in manipulating left end of genome resulted in using wild-type	Loss of manipulability in part 1 (containing A0)
gene 0.4 (1418)	Single base deletion	Unknown	Frameshift after 27th amino acid followed by early termination of gene *0.4*
D6L (1304-1310) **D6R (1494-1500)**	Restriction sites appear twice.	Inefficiency of digestion of scaffold	No expected change
gene 0.6B	Single base addition	Error is known to be in stock of wild-type genome	Dependent upon nature of putative translational slippage in formation of gene *0.6B*
D11L (3302-3307)	Restriction site appears twice	Inefficiency of digestion of scaffold	No expected change
gene 1 (4877)	Single base mutation	Error in PCR or within wild-type genome	Silent mutation, no expected change
gene 1 (5159)	Single base mutation	Error in PCR or within wild-type genome	Silent mutation, no expected change
gene 1 (5399)	Single base mutation	Error in PCR or within wild-type genome	Silent mutation, no expected change
D14R (6591-6597)	Restriction site appears twice	Inefficiency of digestion of scaffold	No expected change
TE (7827)	Single base deletion	Primer synthesis error	Possible loss of function of transcriptional terminator.
D20L (8082-8086)	Restriction site appears twice	Inefficiency of digestion of scaffold	No expected change
U4 (8153-8159)	Restriction site was not added in construction	Failure in site-directed mutagenesis	Loss of manipulability of overlap in parts 18 and 19
D22L (8247-8253)	Restriction site appears twice	Inefficiency of digestion of scaffold	No expected change

Table 7-2. Errors in synthesis & construction of section *beta.*

Location on T7.1 (Genome Position)	Nature of Difference	Probable Reason for Difference	Expected Outcome
gene 1.7 (8794)	Single base silent mutation	Error during PCR or within wild-type genome	No expected change
gene 1.8 (9245)	Single base mutation	Error during PCR or within wild-type genome	Amino acid change in gene 1.8 from Asp to Gly
gene 2.0 (9447)	Single base mutation	Error during PCR or within wild-type genome	Amino acid change in gene 2.0 from Glu to Val
gene 2.5 (10351)	Singe base deletion	Error during primer design	deletion in stop codon; read-through adding on 8AA
gene 2.8 (10627)	Single base mutation	Error during PCR	Amino acid change in gene 2.8 from Asp to Gly
gene 2.8 (10717-10803)	82 base deletion	Error in cloning of part	Loss of function in gene 2.8 in addition to unknown effect on translation of 3.0 due to read-through
gene 3.0 (10926)	Single base silent mutation	Error during PCR or within wild-type genome	No expected change

gene	calculated strength	gene	calculated strength	gene	calculated strength	gene	calculated strength
0.3	7.28	2.8	4.3	*6.7*	7.64	*18.5*	6.9
0.4	5.64	3	5.89	*7*	6.61	*18.7*	7.64
0.5	5.28	3.5	8.32	*7.3*	6.88	*19*	4.89
0.7	4.94	3.8	6.79	*7.7*	5.51	*19.2*	5.33
1	5.59	4.1	6.35	*8*	4.99	*19.3*	5.24
1.1	6.48	4.2	7.91	*9*	6.65	*19.5*	7.01
1.2	7.84	4.3	7.31	*11*	7.8	*0.6A*	5.62
1.3	7.61	4.5	8.33	*12*	6.69	*10A*	7.37
1.4	6.85	4.7	7.34	*13*	6.21	*4A*	6.76
1.5	7	5	8.57	*14*	6.86	*4B*	6.8
1.6	7.13	5.3	6.61	*15*	6.37	B0030	6.13
1.7	6.18	5.5	8.47	*16*	6.55	B0031	3.84
1.8	7.56	5.7	6.18	*17*	5.87	B0032	6.31
2	7.94	5.9	6.69	*17.5*	5.92	B0033	5.87
2.5	6.86	6	7.34				

Table 7-3. Calculated ribosome binding site strength for T7 genes. I used the computational algorithm from the Barrick et al., 1994 study to predict RBS strength of all the T7 genes and 4 BioBrick RBS (B0030-B0033).

Chapter 7.3. Input Files

Chapter 7.3.1. Input Files for Simple Gene Expression Models

Tabasco Input File for Single-molecule Simple Gene Expression model

```
<REQUEST>
<EXECUTE-SIMULATION runs="500" random_seed="34143" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="TRAFFIC_JAM">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="40" id="2" organism_id="2" footprint="15" name="coli Pol" />
<SPECIES name="test" n="0" ID="3" organism="1" />
<REACTIONS>
<REACTION>
3>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="3800" copy_number="1" entry_rate_constant="50000" entry_offsite="3800">
<PROMOTER start="405" stop="428" startsite="423" name="phi0L" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="4e7" aoff="4" ainiton="1.2" runoff_percent="0" aelong="0.23" arecyc="0" />
</PROMOTER>
<TERMINATOR start="3600" stop="3610" stopsite="3610" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
</TERMINATOR>
<RBS start="495" stop="500" startsite="500"  initstepsize="20" strength="1.15e4" stopsite="522" protid="3"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Tabasco Input File for Species-level Simple Gene Expression model

```
<REQUEST>
<EXECUTE-SIMULATION runs="1000" random_seed="34143" simulation_name="test-1"
time_step="50" time_end="25000" min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="TRAFFIC_JAM">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="40" id="2" organism_id="2" footprint="15" name="coli Pol" />
<SPECIES name="rib" n="10000" ID="3" organism="1" />
<SPECIES name="pol" n="700" ID="4" organism="1" />
<SPECIES name="promoter" n="1" ID="5" organism="1" />
<SPECIES name="pol-promoter" n="0" ID="6" organism="1" />
<SPECIES name="pol-promoter-init" n="0" ID="7" organism="1" />
<SPECIES name="pol-dna-elong" n="0" ID="8" organism="1" />
<SPECIES name="rbs" n="0" ID="9" organism="1" />
<SPECIES name="rib-rbs" n="0" ID="10" organism="1" />
<SPECIES name="rib-elong" n="0" ID="11" organism="1" />
<SPECIES name="cust_prot" n="0" ID="12" organism="1" />
<REACTIONS>
<REACTION>
4+5>6;4e7
</REACTION>
```

```
<REACTION>
6>4+5;4
</REACTION>
<REACTION>
6>7;1.2
</REACTION>
<REACTION>
7>5+8;0.23
</REACTION>
<REACTION>
8>4+9;0.645
</REACTION>
<REACTION>
3+9>10;1.15e4
</REACTION>
<REACTION>
10>9+11;0.1308
</REACTION>
<REACTION>
11>3+12;20
</REACTION>
<REACTION>
12>0;7e-4
</REACTION>
<REACTION>
9>0;2.5e-3
</REACTION>
<REACTION>
10>3;2.5e-3
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="3800" copy_number="1" entry_rate_constant="50"
entry_offsite="850">
<PROMOTER start="405" stop="428" startsite="423" name="phi0L" organism_id="1">
       <POLYMERASE polymeraseID="2" aon="0" aoff="4" ainiton="1.2" runoff_percent="35"
aelong="5" arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3600" stop="3610" stopsite="3610" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="0"
stopsite="3500" protid="3" initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Chapter 7.3.2. Input Files for Polymerase Interaction Simulations

Tabasco Input File for Downstream Falloff

```
<REQUEST>
<EXECUTE-SIMULATION runs="100" random_seed="34206" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="DOWNSTREAM_FALL_OFF">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="300" id="2" organism_id="1" footprint="15" name="T7 pol" />
<POLYMERASE n="700" speed="40" id="3" organism_id="2" footprint="15" name="Coli pol" />
<SPECIES name="test-1" n="0" ID="4" organism="1" />
<SPECIES name="test-2" n="0" ID="5" organism="1" />
```

```
<REACTIONS>
<REACTION>
4>0;7E-4
</REACTION>
<REACTION>
5>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="4000" copy_number="1" entry_rate_constant="5000" entry_offsite="3950">
<PROMOTER start="405" stop="428" startsite="423" name="A1" organism_id="2">
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="0.28" ainiton="0.2" runoff_percent="100" aelong="6"
arecyc="0" />
</PROMOTER>
<PROMOTER start="1505" stop="1528" startsite="1523" name="phi10" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="1.26e8" aoff="0.2" ainiton="1.2" runoff_percent="70" aelong="5"
arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3900" stop="3910" stopsite="3910" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
<POLYMERASE ID="3" efficiency="0.99" />
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="1.15e4" stopsite="1499" protid="4"
initRateConstant="0.14" mrnadeg="2.5e-3" />
<RBS start="1585" stop="1600" startsite="1600"  initstepsize="50" strength="1.15e4" stopsite="3600" protid="5"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Tabasco Input File for Upstream Falloff

```
<REQUEST>
<EXECUTE-SIMULATION runs="100" random_seed="34206" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="UPSTREAM_FALL_OFF">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="300" id="2" organism_id="1" footprint="15" name="T7 pol" />
<POLYMERASE n="700" speed="40" id="3" organism_id="2" footprint="15" name="Coli pol" />
<SPECIES name="test-1" n="0" ID="4" organism="1" />
<SPECIES name="test-2" n="0" ID="5" organism="1" />
<REACTIONS>
<REACTION>
4>0;7E-4
</REACTION>
<REACTION>
5>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="4000" copy_number="1" entry_rate_constant="5000" entry_offsite="3950">
<PROMOTER start="405" stop="428" startsite="423" name="A1" organism_id="2">
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="0.28" ainiton="0.2" runoff_percent="100" aelong="6"
arecyc="0" />
</PROMOTER>
<PROMOTER start="1505" stop="1528" startsite="1523" name="phi10" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="1.26e8" aoff="0.2" ainiton="1.2" runoff_percent="70" aelong="5"
arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3900" stop="3910" stopsite="3910" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
<POLYMERASE ID="3" efficiency="0.99" />
```

```
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="1.15e4" stopsite="1499" protid="4"
initRateConstant="0.14" mrnadeg="2.5e-3" />
<RBS start="1585" stop="1600" startsite="1600"  initstepsize="50" strength="1.15e4" stopsite="3600" protid="5"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Tabasco Input File for Traffic Jam

```
<REQUEST>
<EXECUTE-SIMULATION runs="100" random_seed="34206" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="TRAFFIC_JAM">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="300" id="2" organism_id="1" footprint="15" name="T7 pol" />
<POLYMERASE n="700" speed="40" id="3" organism_id="2" footprint="15" name="Coli pol" />
<SPECIES name="test-1" n="0" ID="4" organism="1" />
<SPECIES name="test-2" n="0" ID="5" organism="1" />
<REACTIONS>
<REACTION>
4>0;7E-4
</REACTION>
<REACTION>
5>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="4000" copy_number="1" entry_rate_constant="5000" entry_offsite="3950">
<PROMOTER start="405" stop="428" startsite="423" name="A1" organism_id="2">
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="0.28" ainiton="0.2" runoff_percent="100" aelong="6"
arecyc="0" />
</PROMOTER>
<PROMOTER start="1505" stop="1528" startsite="1523" name="phi10" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="1.26e8" aoff="0.2" ainiton="1.2" runoff_percent="70" aelong="5"
arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3900" stop="3910" stopsite="3910" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
<POLYMERASE ID="3" efficiency="0.99" />
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="1.15e4" stopsite="1499" protid="4"
initRateConstant="0.14" mrnadeg="2.5e-3" />
<RBS start="1585" stop="1600" startsite="1600"  initstepsize="50" strength="1.15e4" stopsite="3600" protid="5"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Tabasco Input File for Traffic Jam with No T7 RNA Polymerase

```
<REQUEST>
```

```
<EXECUTE-SIMULATION runs="100" random_seed="34206" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="TRAFFIC_JAM">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="0" speed="300" id="2" organism_id="1" footprint="15" name="T7 pol" />
<POLYMERASE n="700" speed="40" id="3" organism_id="2" footprint="15" name="Coli pol" />
<SPECIES name="test-1" n="0" ID="4" organism="1" />
<SPECIES name="test-2" n="0" ID="5" organism="1" />
<REACTIONS>
<REACTION>
4>0;7E-4
</REACTION>
<REACTION>
5>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="4000" copy_number="1" entry_rate_constant="5000" entry_offsite="3950">
<PROMOTER start="405" stop="428" startsite="423" name="A1" organism_id="2">
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="0.28" ainiton="0.2" runoff_percent="100" aelong="6"
arecyc="0" />
</PROMOTER>
<PROMOTER start="1505" stop="1528" startsite="1523" name="phi10" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="1.26e8" aoff="0.2" ainiton="1.2" runoff_percent="70" aelong="5"
arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3900" stop="3910" stopsite="3910" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
<POLYMERASE ID="3" efficiency="0.99" />
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="1.15e4" stopsite="1499" protid="4"
initRateConstant="0.14" mrnadeg="2.5e-3" />
<RBS start="1585" stop="1600" startsite="1600"  initstepsize="50" strength="1.15e4" stopsite="3600" protid="5"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Tabasco Input File for Traffic Jam with No *E. coli* RNA polymerase

---

```
<REQUEST>
<EXECUTE-SIMULATION runs="100" random_seed="34206" simulation_name="test-1" time_step="50" time_end="25000"
min_num_for_init_output="1">
<CELL volume="1E-15" growth_rate="0" polymerase_interaction_model="TRAFFIC_JAM">
<RIBOSOME initial_number="10000" speed="40" footprint="35" />
<POLYMERASE n="700" speed="300" id="2" organism_id="1" footprint="15" name="T7 pol" />
<POLYMERASE n="0" speed="40" id="3" organism_id="2" footprint="15" name="Coli pol" />
<SPECIES name="test-1" n="0" ID="4" organism="1" />
<SPECIES name="test-2" n="0" ID="5" organism="1" />
<REACTIONS>
<REACTION>
4>0;7E-4
</REACTION>
<REACTION>
5>0;7E-4
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="4000" copy_number="1" entry_rate_constant="5000" entry_offsite="3950">
<PROMOTER start="405" stop="428" startsite="423" name="A1" organism_id="2">
```

```
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="0.28" ainiton="0.2" runoff_percent="100" aelong="6"
arecyc="0" />
</PROMOTER>
<PROMOTER start="1505" stop="1528" startsite="1523" name="phi10" organism_id="1">
        <POLYMERASE polymeraseID="2" aon="1.26e8" aoff="0.2" ainiton="1.2" runoff_percent="70" aelong="5"
arecyc="0.7" />
</PROMOTER>
<TERMINATOR start="3900" stop="3910" stopsite="3910" name="TE"  organism="2">
<POLYMERASE ID="2" efficiency="0.99" />
<POLYMERASE ID="3" efficiency="0.99" />
</TERMINATOR>
<RBS start="485" stop="500" startsite="500"  initstepsize="50" strength="1.15e4" stopsite="1499" protid="4"
initRateConstant="0.14" mrnadeg="2.5e-3" />
<RBS start="1585" stop="1600" startsite="1600"  initstepsize="50" strength="1.15e4" stopsite="3600" protid="5"
initRateConstant="0.14" mrnadeg="2.5e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>
```

Chapter 7.3.3. Simulation Optimizations for Different Models

Tabasco Input File for Unfit Simulations

```
<REQUEST>
<EXECUTE-SIMULATION runs="1" random_seed="34145" simulation_name="test-1" time_step="5" time_end="1500"
write_DNA="no" write_InitRNA="yes" write_Energy="yes" compDeg="no">
<CELL volume="8e-16" polymerase_interaction_model="CUSTOM">
<RIBOSOME initial_number="10000" speed="45" footprint="40" />
<POLYMERASE n="1800" speed="45" id="2" organism_id="2" footprint="15" name="coli Pol" />
<POLYMERASE n="0" speed="45" id="3" organism_id="2" footprint="15" name="coli Pol-P" />
<POLYMERASE n="0" speed="45" id="4" organism_id="2" footprint="15" name="coli Pol-2.0" />
<POLYMERASE n="0" speed="45" id="5" organism_id="2" footprint="15" name="coli Pol-P-2.0" />
<POLYMERASE n="0" speed="230" id="6" organism_id="1" footprint="15" name="gp1" />
<POLYMERASE n="0" speed="230" id="7" organism_id="1" footprint="15" name="gp1-3.5" />
<SPECIES name="gp0.3" n="0" ID="8" organism="1" />
<SPECIES name="gp0.4" n="0" ID="9" organism="1" />
<SPECIES name="gp0.5" n="0" ID="10" organism="1" />
<SPECIES name="gp0.6A" n="0" ID="11" organism="1" />
<SPECIES name="gp0.7" n="0" ID="12" organism="1" />
<SPECIES name="gp1.1" n="0" ID="13" organism="1" />
<SPECIES name="gp1.2" n="0" ID="14" organism="1" />
<SPECIES name="gp1.3" n="0" ID="15" organism="1" />
<SPECIES name="gp1.4" n="0" ID="16" organism="1" />
<SPECIES name="gp1.5" n="0" ID="17" organism="1" />
<SPECIES name="gp1.6" n="0" ID="18" organism="1" />
<SPECIES name="gp1.7" n="0" ID="19" organism="1" />
<SPECIES name="gp1.8" n="0" ID="20" organism="1" />
<SPECIES name="gp2.0" n="0" ID="21" organism="1" />
<SPECIES name="gp2.5" n="0" ID="22" organism="1" />
<SPECIES name="gp2.8" n="0" ID="23" organism="1" />
<SPECIES name="gp3.0" n="0" ID="24" organism="1" />
<SPECIES name="gp3.5" n="0" ID="25" organism="1" />
<SPECIES name="gp3.8" n="0" ID="26" organism="1" />
<SPECIES name="gp4A" n="0" ID="27" organism="1" />
<SPECIES name="gp4.2" n="0" ID="28" organism="1" />
<SPECIES name="gp4.3" n="0" ID="29" organism="1" />
<SPECIES name="gp4.5" n="0" ID="30" organism="1" />
<SPECIES name="gp4.7" n="0" ID="31" organism="1" />
<SPECIES name="gp5.0" n="0" ID="32" organism="1" />
```

```
<SPECIES name="gp5.3" n="0" ID="33" organism="1" />
<SPECIES name="gp5.5" n="0" ID="34" organism="1" />
<SPECIES name="gp5.7" n="0" ID="35" organism="1" />
<SPECIES name="gp5.9" n="0" ID="36" organism="1" />
<SPECIES name="gp6.0" n="0" ID="37" organism="1" />
<SPECIES name="gp6.3" n="0" ID="38" organism="1" />
<SPECIES name="gp6.5" n="0" ID="39" organism="1" />
<SPECIES name="gp6.7" n="0" ID="40" organism="1" />
<SPECIES name="gp7" n="0" ID="41" organism="1" />
<SPECIES name="gp7.3" n="0" ID="42" organism="1" />
<SPECIES name="gp7.7" n="0" ID="43" organism="1" />
<SPECIES name="gp8" n="0" ID="44" organism="1" />
<SPECIES name="gp9" n="0" ID="45" organism="1" />
<SPECIES name="gp10A" n="0" ID="46" organism="1" />
<SPECIES name="gp11" n="0" ID="47" organism="1" />
<SPECIES name="gp12" n="0" ID="48" organism="1" />
<SPECIES name="gp13" n="0" ID="49" organism="1" />
<SPECIES name="gp14" n="0" ID="50" organism="1" />
<SPECIES name="gp15" n="0" ID="51" organism="1" />
<SPECIES name="gp16" n="0" ID="52" organism="1" />
<SPECIES name="gp17" n="0" ID="53" organism="1" />
<SPECIES name="gp17.5" n="0" ID="54" organism="1" />
<SPECIES name="gp18" n="0" ID="55" organism="1" />
<SPECIES name="gp18.5" n="0" ID="56" organism="1" />
<SPECIES name="gp19" n="0" ID="57" organism="1" />
<SPECIES name="gp19.5" n="0" ID="58" organism="1" />
<REACTIONS>
<REACTION>
12+2>3+11;3.8E7
</REACTION>
<REACTION>
12+4>5+11;3.8E7
</REACTION>
<REACTION>
21+2>4;3.8E7
</REACTION>
<REACTION>
21+3>5;3.8E7
</REACTION>
<REACTION>
4>21+2;1.1
</REACTION>
<REACTION>
5>21+3;1.1
</REACTION>
<REACTION>
25+6>7;3.8E7
</REACTION>
<REACTION>
7>6+25;3.5
</REACTION>
</REACTIONS>
<DNA_SYSTEM name="phage1" genome_length="39937" copy_number="3" entry_rate_constant="70" entry_offsite="850">
<PROMOTER start="405" stop="428" startsite="423" name="phi0L" organism_id="1">
        <POLYMERASE polymeraseID="6" aon="1.82e8" aoff="0.2" ainiton="3.5" runoff_percent="30" aelong="5.8"
arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="498" stop="548" startsite="542" name="A1" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
```

```
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
</PROMOTER>
<PROMOTER start="626" stop="676" startsite="670" name="A2" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
</PROMOTER>
<PROMOTER start="750" stop="800" startsite="794" name="A3" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff=".28" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
</PROMOTER>
<PROMOTER start="1514" stop="1564" startsite="1558" name="B" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
</PROMOTER>
<PROMOTER start="3113" stop="3163" startsite="3157" name="C" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
</PROMOTER>
<PROMOTER start="5848" stop="5871" startsite="5866" name="phi1.1A" organism_id="1">
        <POLYMERASE polymeraseID="6" aon="6E7" aoff="18" ainiton="3.5" runoff_percent="30" aelong="5.8"
arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="5923" stop="5946" startsite="5941" name="phi1.1B" organism_id="1">
        <POLYMERASE polymeraseID="6" aon="1.82e8" aoff="0.2" ainiton="3.5" runoff_percent="30" aelong="5.8"
arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="6409" stop="6432" startsite="6427" name="phi1.3" organism_id="1">
        <POLYMERASE polymeraseID="6" aon="6e7" aoff="18" ainiton="3.5" runoff_percent="30" aelong="5.8"
arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="7778" stop="7801" startsite="7796" name="phi1.5" organism_id="1">
        <POLYMERASE polymeraseID="6" aon="1.82e8" aoff="0.2" ainiton="3.5" runoff_percent="30" aelong="5.8"
arecyc="0.88" />
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</CELL>
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Tabasco Input File for Initial Fit

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arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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<PROMOTER start="750" stop="800" startsite="794" name="A3" organism_id="2">
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arecyc="0"/>
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arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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<PROMOTER start="1514" stop="1564" startsite="1558" name="B" organism_id="2">
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arecyc="0"/>
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<PROMOTER start="3113" stop="3163" startsite="3157" name="C" organism_id="2">
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arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="7778" stop="7801" startsite="7796" name="phi1.5" organism_id="1">
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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<PROMOTER start="21865" stop="21888" startsite="21883" name="phi9" organism_id="1">
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="34566" stop="34589" startsite="34584" name="phi17" organism_id="1">
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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<PROMOTER start="39229" stop="39252" startsite="39247" name="phiOR" organism_id="1">
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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<RBS start="5992" stop="6007" startsite="6007" initstepsize="50" elongstepsize="" strength="1e7" stopsite="6135" protid="13" initRateConstant="0.3" mrnadeg="1.11e-3" />
<RBS start="6119" stop="6134" startsite="6134" initstepsize="50" elongstepsize="" strength="1e7" stopsite="6394" protid="14" initRateConstant="0.3" mrnadeg="2.38e-3" />
<RBS start="6460" stop="6475" startsite="6475" initstepsize="50" elongstepsize="" strength="1e7" stopsite="7554" protid="15" initRateConstant="0.3" mrnadeg="5.56e-3" />
<RBS start="7593" stop="7608" startsite="7608" initstepsize="50" elongstepsize="" strength="1e7" stopsite="7763" protid="16" initRateConstant="0.3" mrnadeg="5.56e-3" />
<RBS start="7776" stop="7791" startsite="7791" initstepsize="50" elongstepsize="" strength="1e7" stopsite="7880" protid="17" initRateConstant="0.3" mrnadeg="4.17e-3" />
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<RBS start="8150" stop="8165" startsite="8165" initstepsize="50" elongstepsize="" strength="1e7" stopsite="8756" protid="19" initRateConstant="0.3" mrnadeg="2.08e-3" />
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<RBS start="9136" stop="9152" startsite="9158" initstepsize="50" elongstepsize="" strength="1e7" stopsite="9856" protid="22" initRateConstant="0.3" mrnadeg="1.19e-3" />
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<RBS start="10690" stop="10705" startsite="10705" initstepsize="50" elongstepsize="" strength="1e7" stopsite="11161" protid="25" initRateConstant="0.3" mrnadeg="1.19e-3" />
<RBS start="11210" stop="11225" startsite="11225" initstepsize="50" elongstepsize="" strength="1e7" stopsite="11590" protid="26" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="11550" stop="11565" startsite="11565" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13265" protid="27" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="12973" stop="12988" startsite="12988" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13326" protid="28" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="13337" stop="13352" startsite="13352" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13564" protid="29" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="13575" stop="13584" startsite="13584" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13853" protid="30" initRateConstant="0.3" mrnadeg="4.17e-3" />
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<RBS start="17124" stop="17149" startsite="17149" initstepsize="50" elongstepsize="" strength="1e7" stopsite="17359" protid="35" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="17343" stop="17358" startsite="17358" initstepsize="50" elongstepsize="" strength="1e7" stopsite="17517" protid="36" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="17489" stop="17504" startsite="17504" initstepsize="50" elongstepsize="" strength="1e7" stopsite="18406" protid="37" initRateConstant="0.3" mrnadeg="2.38e-3" />
<RBS start="18379" stop="18394" startsite="18394" initstepsize="50" elongstepsize="" strength="1e7" stopsite="18507" protid="38" initRateConstant="0.3" mrnadeg="4.17e-3" />
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<RBS start="18860" stop="18864" startsite="18864" initstepsize="50" elongstepsize="" strength="1e7" stopsite="19131" protid="40" initRateConstant="0.3" mrnadeg="5.56e-3" />

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<RBS start="19835" stop="19848" startsite="19848" initstepsize="50" elongstepsize="" strength="1e7" stopsite="20240" protid="43" initRateConstant="0.3" mrnadeg="5.56e-3" />
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<RBS start="27292" stop="27307" startsite="27307" initstepsize="50" elongstepsize="" strength="1e7" stopsite="27723" protid="49" initRateConstant="0.3" mrnadeg="5.56e-3" />
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<RBS start="28302" stop="28317" startsite="28325" initstepsize="50" elongstepsize="" strength="1e7" stopsite="30568" protid="51" initRateConstant="0.3" mrnadeg="0" />
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<RBS start="36329" stop="36344" startsite="36344" initstepsize="50" elongstepsize="" strength="1e7" stopsite="36547" protid="54" initRateConstant="0.3" mrnadeg="0" />
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<RBS start="36902" stop="36917" startsite="36917" initstepsize="50" elongstepsize="" strength="1e7" stopsite="37348" protid="56" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="37355" stop="37370" startsite="37370" initstepsize="50" elongstepsize="" strength="1e7" stopsite="39130" protid="57" initRateConstant="0.3" mrnadeg="5.56e-3" />
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</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>

Tabasco Input File for Increased Promoter Strength Fits

<REQUEST>
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</CELL>
</EXECUTE-SIMULATION>
</REQUEST>

Tabasco Input File for Alternative Degradation Fitst

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</CELL>
</EXECUTE-SIMULATION>
</REQUEST>

Tabasco Input File for DownStream Falloff Fits

<REQUEST>

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12+2>3+11;3.8E7

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</REACTION>
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12+4>5+11;3.8E7
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<REACTION>
21+2>4;3.8E7
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21+3>5;3.8E7
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5>21+3;1.1
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25+6>7;3.8E7
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7>6+25;3.5
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arecyc="0.88" />
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arecyc="0.22" />
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arecyc="0.19"/>
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arecyc="0.19"/>
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arecyc="0.19"/>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
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arecyc="0.22" />
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arecyc="0.22" />
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arecyc="0.88" />
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arecyc="0.22" />
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<RBS start="34609" stop="34624" startsite="34624" initstepsize="50" elongstepsize="" strength="1e7" stopsite="36285" protid="53" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="36329" stop="36344" startsite="36344" initstepsize="50" elongstepsize="" strength="1e7" stopsite="36547" protid="54" initRateConstant="0.3" mrnadeg="0" />
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<RBS start="36902" stop="36917" startsite="36917" initstepsize="50" elongstepsize="" strength="1e7" stopsite="37348" protid="56" initRateConstant="0.3" mrnadeg="3.33e-3" />
<RBS start="37355" stop="37370" startsite="37370" initstepsize="50" elongstepsize="" strength="1e7" stopsite="39130" protid="57" initRateConstant="0.3" mrnadeg="4.17e-3" />
<RBS start="39374" stop="39389" startsite="39389" initstepsize="50" elongstepsize="" strength="1e7" stopsite="39538" protid="58" initRateConstant="0.3" mrnadeg="4.17e-3" />
</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>

Tabasco Input File for Traffic Jam Model Fits

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12+4>5+11;3.8E7
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21+2>4;3.8E7
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21+3>5;3.8E7
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4>21+2;1.1
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5>21+3;1.1
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25+6>7;3.8E7
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7>6+25;3.5
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="498" stop="548" startsite="542" name="A1" organism_id="2">
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arecyc="0.19"/>
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        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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<PROMOTER start="626" stop="676" startsite="670" name="A2" organism_id="2">
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arecyc="0.19"/>
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</PROMOTER>
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        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff=".28" ainiton="0.4" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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</PROMOTER>
<PROMOTER start="3113" stop="3163" startsite="3157" name="C" organism_id="2">
        <POLYMERASE polymeraseID="2" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="100" aelong="6"
arecyc="0"/>
        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff="2.8" ainiton="0.19" runoff_percent="70" aelong="6"
arecyc="0.19"/>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
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arecyc="0.88" />
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        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
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        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
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arecyc="0.88" />
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<POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45" arecyc="0.22" />
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<RBS start="7776" stop="7791" startsite="7791" initstepsize="50" elongstepsize="" strength="1e7" stopsite="7880" protid="17" initRateConstant="0.3" mrnadeg="5.56e-3" />
<RBS start="7891" stop="7906" startsite="7906" initstepsize="50" elongstepsize="" strength="1e7" stopsite="8166" protid="18" initRateConstant="0.3" mrnadeg="5.56e-3" />
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<RBS start="11550" stop="11565" startsite="11565" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13265" protid="27" initRateConstant="0.3" mrnadeg="5.56e-3" />
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<RBS start="13337" stop="13352" startsite="13352" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13564" protid="29" initRateConstant="0.3" mrnadeg="5.56e-3" />
<RBS start="13575" stop="13584" startsite="13584" initstepsize="50" elongstepsize="" strength="1e7" stopsite="13853" protid="30" initRateConstant="0.3" mrnadeg="5.56e-3" />
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<RBS start="14338" stop="14353" startsite="14353" initstepsize="50" elongstepsize="" strength="1e7" stopsite="16467" protid="32" initRateConstant="0.3" mrnadeg="2.78e-3" />
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<RBS start="17489" stop="17504" startsite="17504" initstepsize="50" elongstepsize="" strength="1e7" stopsite="18406" protid="37" initRateConstant="0.3" mrnadeg="4.17e-3" />
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<RBS start="24213" stop="24228" startsite="24228" initstepsize="50" elongstepsize="" strength="1e7" stopsite="24818" protid="47" initRateConstant="0.3" mrnadeg="0" />
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<RBS start="28302" stop="28317" startsite="28325" initstepsize="50" elongstepsize="" strength="1e7" stopsite="30568" protid="51" initRateConstant="0.3" mrnadeg="0" />
<RBS start="30580" stop="30595" startsite="30595" initstepsize="50" elongstepsize="" strength="1e7" stopsite="34551" protid="52" initRateConstant="0.3" mrnadeg="2.38e-3" />
<RBS start="34609" stop="34624" startsite="34624" initstepsize="50" elongstepsize="" strength="1e7" stopsite="36285" protid="53" initRateConstant="0.3" mrnadeg="8.33e-3" />
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</DNA_SYSTEM>
</CELL>
</EXECUTE-SIMULATION>
</REQUEST>

Tabasco Input File for Upstream Falloff Fits

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12+4>5+11;3.8E7
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5>21+3;1.1
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25+6>7;3.8E7
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7>6+25;3.5
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="498" stop="548" startsite="542" name="A1" organism_id="2">
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        <POLYMERASE polymeraseID="3" aon="1.5e7" aoff=".28" ainiton="0.4" runoff_percent="70" aelong="6"
arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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arecyc="0.19"/>
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        <POLYMERASE polymeraseID="5" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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arecyc="0.19"/>
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arecyc="0"/>
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arecyc="0.19"/>
        <POLYMERASE polymeraseID="4" aon="0" aoff="0" ainiton="0" runoff_percent="0" aelong="0" arecyc="0"/>
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arecyc="0.19"/>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
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        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
<PROMOTER start="7778" stop="7801" startsite="7796" name="phi1.5" organism_id="1">
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        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
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        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="18" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
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        <POLYMERASE polymeraseID="7" aon="6e7" aoff="18" ainiton="0.875" runoff_percent="18" aelong="1.45"
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
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arecyc="0.88" />
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arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
</PROMOTER>
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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arecyc="0.88" />
        <POLYMERASE polymeraseID="7" aon="1.82e8" aoff="0.2" ainiton="0.875" runoff_percent="42" aelong="1.45"
arecyc="0.22" />
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</CELL>
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</REQUEST>
